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BIOLOGICAL CONTROL OF WEEDS LABORATORY-EUROPE

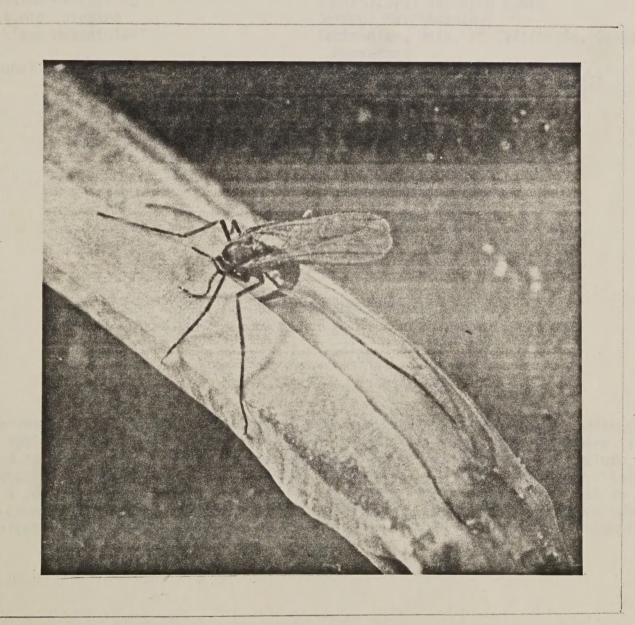
1982 ANNUAL REPORT

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# BIOLOGICAL CONTROL OF WEEDS LABORATORY-EUROPE Rome, Italy

1982 ANNUAL REPORT



Gall midge, ovipositing on leafy spurge

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### INTRODUCTION

The year of 1982 was an exceptional year for the Rome Laboratory because we finished the work necessary to prepare the petitions for the introductions of three very good candidate insects into quarantine at Albany. Two of these insects, <a href="Petrolonche">Pterolonche</a> inspersa and <a href="Bangasternus orientalis">Bangasternus orientalis</a> are natural enemies of yellow starthistle and the third <a href="Bayeria capitigena">Bayeria capitigena</a> is a gall midge that deforms the new growth of leafy spurge.

The work in Greece moved ahead satisfactorily and several natural enemies of both leafy spurge and the <u>Centaurea</u> spp. were brought a step closer to the pre-introductory host testing. Also, the finding in Greece disqualified what we thought was an excellent candidate. It was found, at the end of the work season, that this candidate weevil (<u>Bruchidius tuberculatus</u> Hochh.) will attack safflower seeds.

Using the USDA Rome Laboratory as a meeting place an  $\underline{ad}$   $\underline{hoc}$  group of 20 Italian weed scientists, plant pathologists, and entomologists had their first meeting to organize a program of Biological control of weeds in Italy. Dott. Pasquale Pecora of our laboratory organized and coordinated this meeting.

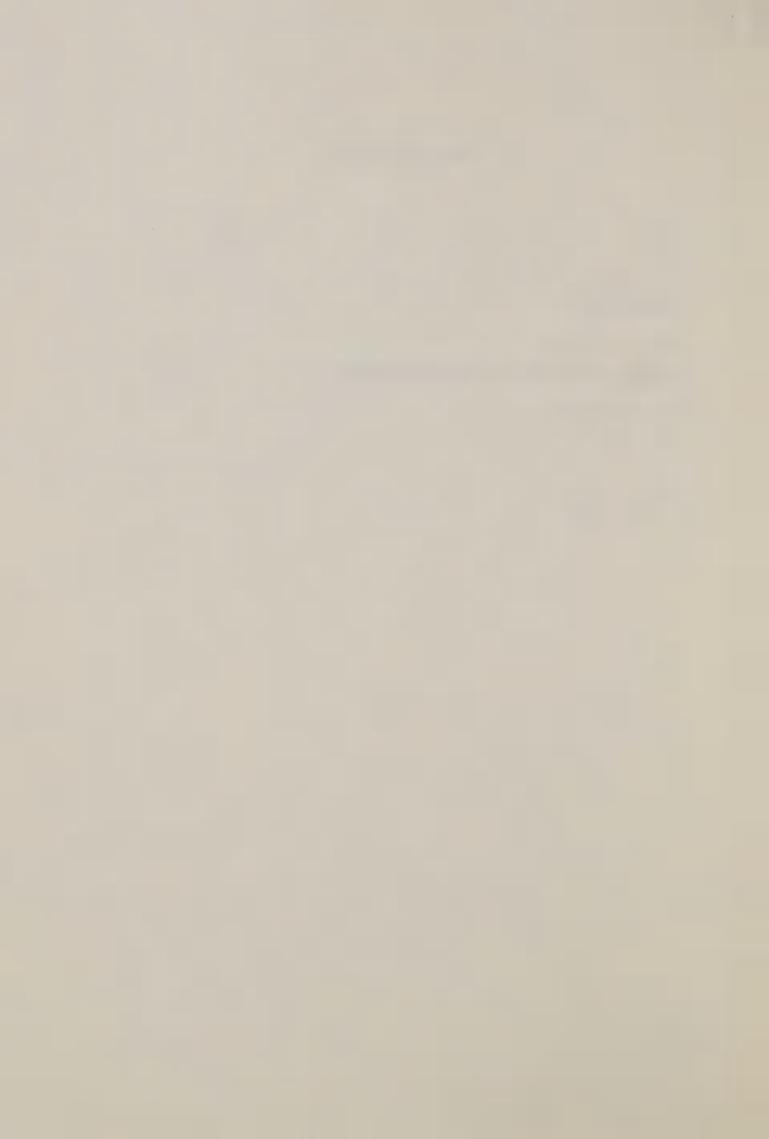
In February, Dr. Stephen L. Clement joined the staff of the Rome Laboratory bringing his expertise in experiment design, his experience in field entomology and a high energy level with him.

Paul H. Dunn

Research & Location Leader

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Euphorbia spp. (Leafy spurge) project.//
A./Rizza, P./Pecora, and M./Stazi (part-time)

# 1) Bayeria capitigena Bremi (Dipt.: Cecidomyiidae)

This insect was screened by USDA Rome Laboratory. All the data gathered thus for have been included in the attached petition which will be submitted for the clearance of the midge into quarantine for testing on U.S. native spurges and other plants.

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A petition for the introduction into quarantine for further testing of <u>Bayeria capitigena</u> (Bremi) (Diptera: Cecidomyiidae), a potential biocontrol agent of <u>leafy spurge</u> (<u>Euphorbia esula-virgata</u> "complex").

Prepared by:

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### I. Introduction

Leafy spurge (<u>Euphorbia</u> <u>esula-virgata</u> "complex") is a weed of European origin that has become a serious problem in pastures, ranges and non-cropland areas in North America. On rangelands and pastures it becomes dominant, displacing useful forage plants. It is a poisonous plant that produces an irritant that causes dermatitis to men and animals (Kingsburg 1964). Cattle usually refuse leafy spurge as food unless it is given to them in weedy hay or if better forage is unavailable. According to Dunn (1979), leafy spurge occurs in 25 states with 451 infested counties.

A conservative estimate of loss in the U.S., in terms of expenditure for controlling leafy spurge and lost of productivity, is \$10.5 million annually (Noble et al. 1979).

The area of worst infestation in North America is defined by a 1,200 mile-diameter circle, centered near Wolf Point, Montana. This area covers parts of 5 states and 5 Canadian provinces and encompasses nearly 2.5 million acres. In the U.S., Minnesota has the highest infestation at 800,000 acres, followed by North Dakota and Montana with 600,000 and 543,000 acres, respectively (Noble et al. 1979). The problem is most severe on undisturbed lands. Even on cultivated cropland areas where leafy spurge has been controlled, it can reduce crop yields from 10 to 100% (Darscheid and Wrage, 1972).

This alien weed is continuing to spread at an alarming rate. The weed is difficult and expensive to control by cultural, mechanical, and chemical means, or combinations of these methods.

Because of its foreign origin and the large number of natural enemies associated with it in Eurasia, leafy spurge is considered to be an excellent candidate weed for biological control.

A program for biological control of leafy spurge was started by the U.S. Department of Agriculture in 1973. Efforts by the C.I.B.C. have resulted in the release of three insect species (2 moths and a beetle) in North America. The moths, a defoliator (Hyles euphorbia L., Lepidoptera: Sphingidae) and a root borer (Chamaesphecia tenthrediniformis Den. & Shiff., Lepidoptera: Sesiidae), failed to become established on Euphorbia esula-virgata complex in Canada and the U.S., following their release in 1974 (Baker and Anderson, 1974) and 1977 (Dunn personal communication), respectively. A longhorned beetle (Oberea erythrocephala Schrank, Coleoptera: Cerambycidae) has been periodically released between 1980 and 1982. To avoid some of the problems experienced with the 2 moths, Schroeder (1980) tested O. erythrocephala on U.S. biotypes of leafy spurge during the initial phase of his research with this beetle.

Efforts to find additional biological control agents which could reduce the density of leafy spurge in North America led us to focus on the potential of a gall midge, <u>Bayeria capitigena</u> (Bremi) (Diptera: Cecidomyiidae). Because this insect appeared to stress <u>Euphorbia</u> spp. in the field we undertook a study to determine its host specificity and effectiveness. To that end, we conducted in-depth studies in the field and at the USDA Laboratory, Rome, Italy from 1981-1982.

### II. Taxonomic position

The genus <u>Bayeria</u> is in the family Cecidomyiidae (Diptera), subfamily Cecidomyiinae, supertribe Oligotrophidi, and tribe Poomyiini (Rubsaamen and Hedicke 1925-39).

The species was described by Bremi (1847) as <u>Cecidomia capitigena</u>. Later, this species was placed in the genus <u>Perrissia Rondani</u> (Kieffer J. 1898). Rubsaamen (1914) erected the genus <u>Bayeria</u> to include an undetermined species that caused deformations to the apical tips of <u>Euphorbia characias</u> L. Finally, Rubsaamen and Hedicke (1925-39) placed <u>capitigena</u> in the genus <u>Bayeria</u>; they also provided descriptions of the galls and adults. The larva of <u>B. capitigena</u>

(Bremi) was described by Mohn (1955). (See footnote)\*

Besides B. capitigena, there are 3 other species in the genus: B. erysimi Rubs., B. thymicola (Kieffer) and B. salicariae (Kieffer). Buhr (1965) gives the following host plants for B. erysimi: Erysimum canescens Roth, E. cheiranthoides L., E. crepidifolium Rchb., E. hieracifolium L., E. pannonicum Crantz, and E. repandum L.. Houard (1908, 1909, 1913) gives the following host plants for B. thymicola: Thymus vulgaris L., T. striatus Vahl, T. serpyllum L., T. ovatus L., T. chamaedrys Fries, T. mastichina L., T. angustifolium Pers. var. normalis Rouy, and T. montanus Waldst & Kit.; in addition, Houard (1917) gives T. ciliatus Desf.. Lythrum salicaria L. is the only reported host plant of B. salicariae (Kieffer, 1888).

The genus Bayeria has a Palearctic distribution (Barnes, 1949, Houard, 1908,

1909, 1913, Kieffer 1888).

According to some of the contemporary authorities on gall midges (Skuhrava and Skuhravy 1973; Nijveldt 1969; Solinas, pers. comm.), the taxonomic status of this group is not clear because of the great variability in morphological characteristics. Recent studies have shown that many of the earlier species

descriptions are now considered to be invalid.

Professor M. Solinas, a specialist at the University of Bari, provided assistance in identifying the gall midges on <u>Euphorbia</u>. Our material, collected from <u>E. esula</u> and <u>E. cyparissias</u>, was repeatedly identified by him as <u>Bayeria capitigena</u> (causes a meristematic tip gall) or <u>Dasineura capsulae</u> (Kieffer) (causes a fruit gall). According to Solinas the larvae and adults of <u>Bayeria</u> and <u>Dasineura can be distinguished</u> by the following characters:

Bayeria:

Larva: the anal papillae are immersed in the tegumental verrucae.

Adult: in the male genitalia, the distal clasp segments are completely pu-

bescent.

Dasineura:

Larva: the anal papillae are located in the middle of a small, smooth and

convex area.

Adult: in the male genitalia, the distal clasp segments are pubescent only

in the basal part.

# III. Geographic distribution

B. <u>capitigena</u> is widely distributed in Europe (Kieffer 1888). We have found this midge in Italy, Austria, Hungary, Rumania, Bulgaria and Greece during our surveys to discover natural enemies associated with Euphorbia spp.

<sup>\*</sup> The species has been placed in <u>Bayeria</u> but R.J.Gagne, Systematic Entomology Lab., IIBIII, ARS,USDA does not consider the name to apply to the <u>Dasineura spp.</u> on <u>Euphorbia spp.</u>

# IV. Host plants

A review of the literature showed that  $\underline{B}$ .  $\underline{capitigena}$  is associated with  $\underline{Euphorbia}$  spp. The following species are given as host plants:  $\underline{E}$ .  $\underline{esula}$  L.,  $\underline{E}$ .  $\underline{cyparissias}$  L.,  $\underline{E}$ .  $\underline{dulcis}$  L.,  $\underline{E}$ .  $\underline{virgata}$  W. et K.,  $\underline{E}$ .  $\underline{verrucosa}$  L.,  $\underline{and}$   $\underline{E}$ .  $\underline{amygdaloides}$  L. (Bohr 1965). In addition we have collected this midge on  $\underline{E}$ .  $\underline{lucida}$  W. et K. and on  $\underline{E}$ .  $\underline{platyphyllos}$  L. in Italy.

# V. Life history

# a) Field Biology

- Materials and Methods - Specific aspects of the biology of B. capitigena were studied in 1982 at San Rossore (Pisa, Italy), a presidential hunting pre-

serve, where  $\underline{E}$ .  $\underline{esula}$  and  $\underline{E}$ .  $\underline{cyparissias}$  are relatively abundant.

Daily activity periods of adults were determined by censusing 50 plants of E. esula on 3 days in 1983 (May 27, June 16, July 3). On each date, 50 plants were checked 3 times a day; in the morning (7:00-9:00AM), in the warmer hours (1:00-3:00PM) and for 2 hours at sunset. Also, young meristematic tips were checked for eggs on May 27 and June 16. Each time, 50 meristematic tips were randomly collected and then examined with a stereomicroscope to record the number of eggs/tip.

Twenty-five galls were randomly collected on E. esula on each of the following days: May 11, May 27, June 18, July 3, July 16, August 27 and October 2, 1982. These galls were dissected and the number of alive and dead larvae, and the

number of pupae was recorded.

- Results - Adults were seen flying around the host plant in the morning or around sunset. During the warmer hours of the day, the adults usually re-

treated to sheltered areas in the grass.

The females select the external and internal leaves of the meristematic tip to lay eggs. The mean (+ SD) number of eggs/tip (n = 50) collected on May 27 was 28.16+19.45; eggs were found on 24% of these tips. On June 16 this figure was 37.50+15.12 eggs/tip; eggs were found on 20% of the tips. The eggs were laid in groups: 18.15+6.91 eggs/group (n = 27; range 8-25 eggs/group).

Fewer numbers of larvae were found in the mid-July and late- August collections of 25 galls (Table 1). Also, mortality was 6% in May and increased to 31% in July and 28% in August. From dissections of these galls we also found that newly hatched larvae moved to the internal part of meristematic tips to feed on young leaves. Furthermore, it was found that pupation occurred inside galls with each larva spinning a thin silk cocoon (2-3 mm in length) around itself before pupating. The pupae were usually found in groups. For example, there were 6.40+3.14 pupae in 22 groups (range of 1-12 pupae/group). There can be more than one group of pupae in a gall. The adults must tear the cocoon to emerge.

B. capitigena overwinter as mature larvae in the soil and pupation occurs in the spring (Jauffret 1973). We assume that the first adults of the 1982 season at S. Rossore emerged around early April because we found the first galls with larvae during the last week of April and the first days of May. Our observations suggest the midge needs about a month to complete its life cycle. New galls containing larvae of various instars can be found in S. Rossore until late October. This probably means that B. capitigena has 5 or more generation/year. The number of generations is probably regulated in

part by weather conditions.

# b) Laboratory Biology

- Materials and Methods - To study the biology of  $\underline{B}$ . capitigena in captivity, a sample of 50 galls was taken from  $\underline{E}$ . esula on each of 7 days (May 11, May 27, June 18, July 2, July 16, August 27, and October 2). This material was returned

to the Rome Laboratory where these studies were conducted.

To determine adult emergence of B. capitigena and its parasitoids from these samples, the galls were placed in an acrylic plastic container (L = 30 cm; W = 16 cm; H = 20 cm) capped with nylon screen. Two cm of sphagnum moss was placed on the bottom of each container and this was moistened with 2-3 cc of water twice weekly. Containers were checked daily to record emerging adults and

parasitoids.

Adult midges emerging from the above containers were then placed in acrylic plastic cages (H = 13 cm;  $\emptyset$  = 9 cm). Cages were capped with nylon screen with the bottom fitted snugly over a 1.5-2 cm thick cork that had a central hole. The hole accomodated a screw top vial ( $\emptyset$  = 1.5 cm; H = 8.5 cm) containing water; 3-4 stems of leafy spurge were wrapped in cotton and placed in the vial. With this technique we were able to collect data on oviposition period, total number of eggs deposited/cage, egg hatch, and adult longevity. All females placed in these cages had emerged on the same day. The number of adults/cage varied as shown in Table 2.

To collect data on percent of egg hatch and the period of eclosion, a sample of newly laid eggs were collected daily between May 20 and May 25 and placed on the top of a layer (2 cm thick) of moist plaster of Paris fitted to the bottom of a 35 cc plastic cup. Egg hatch was recorded daily. Data on percent egg hatch was also collected from eggs laid later in the season (see Table 2).

All of the material for the above laboratory studies was kept in an outdoor insectary. From April to October, when these studies were conducted, the

temperatures ranged from 8°C to 37°C and the RH from 20 to 80%.

In another study, eggs laid by caged adults were transferred to the meristematic tips of 5 potted plants of E. esula. Thirty mature eggs were placed on each of 5 meristematic tips/plant. At 2 to 5 day intervals one plant was randomly selected and its 5 tips were dissected and larvae were placed in 70% ETOH. This method allowed us to collect data on the number of instars and on larval development time. To determine pupal development time, the cocoons from the plant dissected on June 21 were placed in 35 cc plastic containers held in an outdoor insectary; these containers were checked daily for adult emergence. This study started on June 4 and ended on June 27. It was conducted in a shaded outdoor area during which time temperatures ranged from  $10^{\circ}$  to  $33^{\circ}$ C and RH from 30 to 80%. To prevent any interference by feral adults of Bayeria and to exclude parasitoids, a transparent plastic cylinder ( $\emptyset$  = 20 cm; H = 60 cm), capped with nylon organdy, was placed over each potted plant. Two holes ( $\emptyset$  = 10 cm), covered with nylon screen, were cut in the walls of each cylinder to permit aeration.

- Results - In the study designed to record adult emergence from a series of galls collected at S. Rossore the following results were obtained:

Collection date	Number of ad	Number o	f parasitoids
	0	0	
May 11	45	49	_
May 27	30	38	-
June 18	25	28	10
July 3	16	13	15
July 16	4	6	82
August 27	34	24	7
October 2	4	7	_

The hymenopteran parasitoids were identified as Tetrastichus spp. (Eulophidae)

by Dr. E. E. Grissell, USDA, Systematic Entomology Laboratory.

The biological data (Table 2) obtained in the laboratory give some indication of female fecundity and percent egg hatch. For example, adults from the May 11 sample laid more eggs than those which emerged later. Likewise, the highest percentage of egg hatch was seen in May. The oviposition period, expressed as the mean (+ SD) number of days in which eggs were found in cages, was  $2.50\pm0.67$  days (n = 12 cages).

Eggs which were laid between May 20 and May 25, and kept outdoors, hatched in  $4.77\pm1.08$  days (n = 2,223); percent of egg hatch was  $86.00\pm6.56$ . The female longevity was  $3.41\pm0.98$  days (n = 48). Males lived  $2.61\pm2.01$  days (n = 53).

The following results were obtained in the study designed to generate infor-

mation on the number of instars and on larval development time:

Dissection time	<u>I instar</u>	II instar	III instar	Pupae
June 9	100	_	_	-
June 11	66	16	-	
June 14	-	53	9	-
June 17	-	5	50	10
June 21	-	-	10	69

The pupal stage lasted  $4.22\pm0.81$  days (n = 65). Larval instar, pupal, and adult measurements of B. capitigena are shown in Table 3.

# c) Description of life stages

Egg - Newly laid eggs are light red; turning darker with age. The chorion is smooth and soft. Shape: fusiform, slightly curved with rounded ends.

<u>Larva</u> - There are 3 instars. A full grown larva possesses on the ventral side of the first thoracic segment a chitinized structure (sternal spatula). This structure ends in a free anterior extremity which is bidentate.

Pupa - Light red, except for the leg and wing appendages which are reddish-brown.

Adult - Females and males are easily distinguishable. The female has a tapering abdomen that exposes an ovipositor. In the male the terminal part of the abdomen has a pair of forceps.

# VI. Mortality factors

We have discussed this aspect above in the section on field and laboratory biology but we offer some additional comments here. While it appears that larval mortality was mainly due to parasitism, an unknown percentage of larvae may die as a result of density independent mortality factors (ie. high summer temperature).

No pathogenic viruses, bacteria, fungi, protozoa or nematodes were found in 80 larvae and 40 pupae. This material was collected in S. Rossore and shipped to Albany, California, where it was examined by a Consulting Diagnostic Service.

# VII. Effects of B. capitigena on host plants

As a consequence of the attack of  $\underline{B}$ . capitigena leafy and cypress spurge plants at S. Rossore often produced secondary shoots which arose from the basal part of the old meristematic tip. These secondary shoots were often attacked by a subse-

quent generation of adults.

To obtain some data on field infestation levels, samples were taken on July 10, August 27 and October 2. On each date all the infested and uninfested meristematic tips of leafy spurge (either on primary or secondary shoots) were counted in 4 rectangular plots (each 1 m by 50 m). These plots cut across open fields supporting good infestations of leafy spurge. What we discovered was that infes-

tation levels ranged between 10 and 40%.

As for the effect of  $\underline{B}$ . capitigena on individual plants, Jauffret (1973) states: " $\underline{B}$ . capitigena causes the meristematic tips of  $\underline{E}$ . cyparissias to become flat and later flabby because degeneration of the cellular tissue layer takes place. The nutritive tissue suffers a general hypertrophy extended to the cells, to the nucleus and nucleolus. Furthermore, this tissue has a low content of reserves (starch and lipids). The symptoms of the cellular degeneration are precocious. The nutritive cells lose their meristematic character. A renewal of meristematic activity was never observed in  $\underline{B}$ . capitigena infested tips."

### VIII. Potential control value

Using the system developed by Harris (1973) to determine the value of a potential biological control agent, we arrived at a score of 24. This score falls in the category of those agents having good prospect as a biological control agent of weeds.

# Effectiveness score of B. capitigena.

1.	Host specificity	3
2.		1
3.	Indirect damage inflicted	0
4.	Phenology of attack	4
5.	No. of generation	4
6.	No. of progeny/generation	0
7.	Extrinsic mortality factors	4
8.	Feeding behavior	2
9	Compatibility	2
10.	Distribution	4
11.	Effectiveness	0
12.	Size	0

Total

# IX. Host specificity tests

Test plants

To establish the host plant range of  $\underline{B}$ . capitigena, tests were conducted in 1982 with 58 plant species or varieties in 22 families. Included in this spectrum were species closely related to  $\underline{Euphorbia}$  (order  $\underline{Euphorbia}$  or other orders of the superorder Rosidae), representative economic plants in the Rosidae, and host

24

plants of other species of Bayeria. Heywood's Flowering Plants of the World

(1978) was used as a guide in constructing our test list.

Plants or seeds of U.S. biotypes of leafy spurge were provided by the USDA Biological Control of Weeds Laboratory, Albany, California. The remaining plants (or seeds) in the test list were obtained from botanical gardens or commercial seed companies.

### - Materials and Methods -

### Larval survival test

Thirty plants of <u>E</u>. <u>esula</u> with about 100 <u>Bayeria</u> galls <u>in</u> <u>toto</u>, were collected on May 14 at S. Rossore , transplanted into plastic pots ( $\emptyset$  = 22 cm) and transported to the Rome Laboratory. At Rome 10 of these infested plants were transplanted into each of 3 larger plastic pots (55 cm diam; 45 cm height) containing 20 uninfested <u>E</u>. <u>esula</u> plants. Each pot, was then caged by a cylinder of nylon organdy ( $\emptyset$  = 50 cm; H = 70 cm). Plants on each pot were examined daily for evidence of oviposition. By May 20 emerging adults had oviposited on over 50% of the new apical tips. These eggs were used to start the larval survival test.

Each of the 58 plants in the test received 100 eggs distributed over 5 meristematic tips (20 eggs/tip). Usually each test plant species was represented by only one plant. In some instances,  $\underline{E}$ . lathyris for example, five plants were used because each potted plant only had one meristematic tip. A transparent plastic cylinder, 20 cm H and 20 cm  $\emptyset$ , was placed over each potted test plant.

During this test, outdoor temperatures ranged between 9°C and 30°C, and RH

was 30-90%.

As a check on the % of egg eclosion, 200 eggs from the same stock used for this test were equally divided between two 35 cc plastic cup eclosion cages, and held outdoors, where they were monitored daily for hatch.

This test started on May 20 and ended on June 10 when the meristematic tips

were dissected and the number of surviving larvae and pupae was recorded.

### Multiple Choice Test

On May 27, 200 infested plants of E. esula were collected at S. Rossore and transported to the Rome laboratory in plastic pots ( $\emptyset$  = 22 cm). We counted

800 galls on these plants.

This test was conducted in a shaded area (4m x 3.8m) on the grounds of the Rome laboratory. The experimental design involved interspersing infested potted plants containing mature larvae and pupae among test plants in this area. This created a situation where adults on emerging would have the opportunity to select a number of test plants to oviposit on.

There were three plots within the experimental area. Each plot had 120 potted plants: 26 pots of potted E. esula, each with 10 mature galls, and 2 pots/test species for a total of 94 pots. These were arranged in 10 rows each with 12 pots. Pots within a row were separated by 10 cm; there was 20 cm between rows. A distance of 4 m separated plots. All the plants were

randomly assigned in the plots.

On June 1 the first adults were observed to emerge from infested plants. Thus, the test was set up on June 2. Each test plant was checked daily for 10 days for eggs. On June 26, all meristematic tips showing signs of attack by Bayeria were dissected to record the number of dead and alive larvae, and pupae. At this time the number of exposed meristematic tips/test plant were

also counted.

- Results -

Larval survival test

Of the 200 eggs set aside as a check on egg eclosion 92% hatched within 4 days. The results in Table 4 show that this insect completed its life cycle on 11 of 58 test plants. These 11 test plants are all in the genus Euphorbia (subgenus Esula). Besides the control, the Nebraska, Montana, North Dakota, Oregon, and Saskatchewan (Canada) biotypes of leafy spurges served as suitable hosts. The midge also completed its development on E. cyparissias, E. lathyris ("Castro Valley" and "Chico", California varieties), E. peplus, and E. characias.

Multiple choice test

Based on our experience with this midge, 5 or 6 adults (on the average) will emerge from a mature gall. Thus, 1300-1500 adults of B. capitigena could have emerged from the infested plants in each plot. This high population oviposited on plants in the genus Euphorbia (subgenus Esula). (Table 5). Larval development occurred on all of these test plants on which oviposition occurred, except E. characias.

The results shown in Table 5 follow the same basic pattern shown in Table 4, with the exception that in this experiment the midges oviposited and completed larval development on E. myrsinites and E. serrulata (both subgenus Esula).

# X. Discussion

While some gall midges have a wide host plant range, others are restricted to one host (Nijveldt 1969). The genus <u>Bayeria</u> belongs to the latter group, and the candidate insect has been recorded only on plants in the genus <u>Euphorbia</u>.

Our studies reinforce the literature records for this insect.

Because B. capitigena is heavily parasitized in the field at S. Rossore we believe that once released in the U.S. in the absence of its parasitoids the midge would have an even greater potential to attack E. esula. Moreover, because the insect is widely distributed in Europe, the possibility exists that other European biotypes from a variety of areas climatically compatible with specific U.S. and Canadian environments could be introduced.

Using the Harris (1973) scheme for rating the potential of biocontrol agents, we arrived at a score of 24 for <u>B</u>. <u>capitigena</u>. This value falls between 19 and 25, two scores assigned to <u>Ceuthorhynchus litura</u> (F.) and <u>Zeuxidiplosis giardi</u> (Kieff), respectively. These are two insects with good potential as biocontrol

agents (Harris 1973).

We expect this insect to reduce the seed production of leafy spurge, which could play an important part in reducing the rate of spread of this alien weed. The target weed will also come under additional stress because of attacks by

B. capitigena.

Our tests show that E. lathyris is a suitable host for the midge. Calvin (1974, 1977, 1978, 1979) has presented a case for the use of this plant to produce hydrocarbons. We emphasize, however, that the possible use of this plant as a source of fuel to replace petroleum products has not been borne out by the results of field studies (Sachs et al. 1981). Furthermore, Ward (1982) of the Division of Natural Resources and Energy, United Nations, New York, recently reviewed the status and prospects of using E. lathyris as a source of

energy. He concluded that Calvin is overly optimistic and that "further allocation of resources to explore the use of  $\underline{E}$ .  $\underline{lathyris}$  is not warranted". Thus, there does not appear to be any real conflict of interest in terms of the potential attack of  $\underline{E}$ .  $\underline{lathyris}$  by  $\underline{B}$ . capitigena.

U.S. native Euphorbia spp. will be tested in the Albany quarantine facility if

approval is granted for the introduction of this insect into this facility.

# XI. Summary

The following points suggest that  $\underline{B}$ .  $\underline{capitigena}$  warrants serious consideration for approval for introduction into quarantine where additional host specificity tests will be conducted:

1) Literature host records indicate that  $\underline{B}$ . capitigena is associated with the genus Euphorbia.

2) There are no literature records of host plants of economic importance.

3) Based on host specificity studies in Rome, Italy (multiple choice test and larval survival test),  $\underline{B}$ . capitigena has a host range restricted to the genus Euphorbia (subgenus Esula).

4) U.S. biotypes of leafy spurge were found to be suitable hosts.

5) According to the scoring system proposed by Harris, B. capitigena has good potential as a biocontrol agent.

6) Since it occurs over a wide climatic range, different ecotypes could be

introduced in North America.

7) The possible use of <u>Euphorbia lathyris</u> as a source of energy "has not been borne out either by field studies or techno-economic analysis" (Ward 1982). Thus, there is less concern for a possible conflict of interest involving the biocontrol of the target weed.

# XII. Figures and tables



Fig. 1. Mature meristematic tip gall on  $\underline{E}$ .  $\underline{esula}$  caused by  $\underline{B}$ .  $\underline{capitigena}$  larvae.



Fig. 2. Picture shows an old gall on a primary meristematic tip and new galls on secondary tips on  $\underline{E}$ .  $\underline{esula}$ .

Collection date	Number galls	No. galls with larvae and pupae	No. galls with larvae only pupae only	No. galls with pupae only	No. galls No. empty liv	No. Tive Tarvae	No.dead larvaea/ (%)	Total pupae	larvae/	$x + SD^{\underline{b}/}$
May 11	25	17	2	4	2	77	5(6)	142	3.24+3.07 5.68+4.51	5.68+4
May 27	25	15	ഗ	ω	₽.	142	8(5.3) 155	155	5.80+6.10 6.20+5.80	6.20+5.
June 18	25	10	12	2		98	25(20.3)	48	4.76+3.49 1.92+2.36	1.92+2.
July 3	25	14	4	4	ω	127	30(19.1)	69	6.28+7.70 2.76+2.66	2.76+2
July 16	25	4	ڻ ر	2	14	48		6	0.66+1.12 0.60+1.15	0.60+1.
August 27	25	4	2	Ξ	œ	ڻ ن	2(28.5) 71	71	0.26+0.53 2.88+3.10	2.88+3.
October 2	25	2	17	1	6	65	16(19.7)	2	4.84+8.65 0.08+0.02	0.08+0.

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= /q <u>a/</u> =  $\bar{x}+SD$  (n = 25 galls). The percentage of larval mortality is based on total number of larvae found.

Table 2. Biological data on B. capitigena obtained in the laboratory as a result of collecting 50 galls on 7 dates in S. Rossore, Italy, 1982.

Collection	Date emerged	Adult	cs/cage	Total eggs	No.eggs
date		Ŷ	8	laid	hatching (%)
May 11	V/19 V/20 V/21	6 6 7	7 9 8	735 1,045 890	588(80) 888(85) 827(93)
May 27	VI/8	3	4	133	100(75)
June 18	VI/24 VI/25 VI/28 VI/29	2 3 9 3	5 7 4 2	150 147 282 100	80(53) 88(60) 160(57) 55(55)
July 3	VII/8	2	4	90	20(21)
July 16	VIII/2	6	4	136	10(7)
August 27	IX/3	4	8	125	32(25)
October 2	X/13	3	3	120	47(39)

Table 3. Length and width measurements of life stages of <u>Bayeria capitigena</u>, laboratory study, Rome, Italy, 1982.

Life stage and head capsule	Sample	Length (mm)	(mm)	Width (mm)	(mm)
incasur eu	\$1Ze	×1 + SD	Range	x + SD	Range
Egg	50	0.349+0.013	0.340-0.380	0.088+0.007	0.080-0.100
Larval Head Capsule(I instar)	22			0.035+0.003	0.032-0.039
Larval Head Capsule(II instar)	20			0.060+0.005	0.055-0.063
Larval Head Capsule(III instar)	20			0.076+0.003	0.071-0.079
Larva I instar	22	0.470+0,117	0.360-0.680	0.706+0.020	0.060-0.120
Larva II instar	20	1,378+0.376	0.920-1.800	0.287+0.067	0.200-0.400
Larva III instar	20	2.580+0.290	2.000-2.840	0.583+0.069	0.520-0.720
Pupa	15	1.840+0.140	1.360-2.040	0.690+0.060	0.600-0.800
Adult 9*	15	1.900+0.060	1.760-1.940	0.410±0.020	0.380-0.460
Adult 8*	15	1.860+0.210	1.600-2.000	0.420+0.189	0.400-0.440

Measurements are exclusive of antennae.

Table 4. Results of larval survival test with Bayeria capitigenad, Rome, Italy, 1982.

	Test Plants	Alive Larvae	z + SDa/ Dead Laevae	Pupae	% survivalb/
	EUPHORBIACEAE				
	Euphorbia esula L. (Pisa, Italy) Control	1.40+1.34	1.80+1.79	8.40+5.41	49.00+29.03
	E. esula-virgata "complex" Nebraska type	1.40+1.95	1.80+1.30	8.00±3.94	47.00+14.02
	E. esula-virgata "complex" Montana type	3.20+3.11	4.00+2.01	3.60+3.51	34.00+24.08
	E. esula-virgata "complex" North Dakota type	3.20+4.43	1.00+1.41	5.20+4.15	43.00+27.29
	E. esula-virgata "complex" Oregon type	3.20+2.39	3.40+2.97	3.60+3.58	34.00+21.03
20 -	E. esula-virgata "complex" Saskatchewan type	The apical tips wer for galls to be for	The apical tips were completely destroyed by the larvae making it impossible for galls to be formed.	y the larvae mal	king it impossible
	E. cyparissias L. (Pisa, Italy)	· 5.80 <u>+</u> 2.16	4.40+1.34	4.40+2.30	40.00+18.71
	E. lathyris L. "Castro Valley" variety CA.	3.60+2.61	5.00+2.12	2.40+2.51	30.00+17.32
	E. lathyris L. "Chico" variety CA.	1	2.00±3.08	4.20+4.27	21.00+21.33
	E. peplus L. (Palermo, Italy)	2.40+2.51	4.75±1.25	6.00+5.10	42.00+16.81
	E. characias L. (Trieste, Italy)	1.80+2.50	6.20+2.17	1	9.00+12.45
	E. myrsinites L.				

E. pulcherrima Willd. ex Kl.

E. antisiphylitica Zucc.

E. marginata Pursh.

E. serrulata Thuill.

E. milii Ch. des Moulins

E. tirucalli L.

Ricinus communis L.

Mercurialis annua L.

Codiaeum variegatum (L.) Blume var. pictum (Lodd.)

Manihot palmata Mull Arg.

Pedilanthus tithymaloides (L.) Poit.

RUTACEAE

Ruta graveolens L.

LINACEAE

Linum narbonense L.

GERANIACEAE

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Geranium rotundifolium L.

Erodium glutinosum L.

Pelargonium zonale (L.) L'Her.

VITACEAE

Vitis vinifera L.

ROSACEAE

Rosa sp.

UMBELLIFERAE

Daucus carota subsp. sativus (Hoffm)

FABACEAE

Phaseolus vulgaris L.

Medicago sativa L.

ONAGRACEAE

Clarkia elegans Dougl.

APOCYNACEAE

Nerium oleander L.

Vinca major L.

ASCLEPIADACEAE

Asclepias curassavica L. Asclepias syriaca L.

SOLANACEAE

Solanum tuberosum L.

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CONVOLVULACEAE

Ipomoea batatas (L.) Lam cv. Garnet

LABIATAE

Salvia splendens Ker. Gavl.

Thymus serpyllum L.

SCROPHULARIACEAE

Antirrhinum majus L.

COMPOSITAE

Lactuca sativa L. cv. Great Lakes

Cichorium intybus L.

Cynara scolymus L.

han

tin

Snin

CV.

Helianthus tuberosus L.

Sonchus arvensis L.

GRAMINEAE

Zea mays L. - Field corn (B73 x M017)

Zea mays L. - Sweet Corn - (Golden hybrid blend)

LILIACEAE

Lilium sp. cv. Tabasco

BRASSICACEAE

Brassica oleracea L. Capitata Group

MORACEAE

Ficus carica L.

Ficus elastica Roxb. ex Hornem.

CHENOPODIACEAE

Beta vulgaris L. cv. Early wonder

RANUNCOLACEAE

Anemone sp.

a/  $\bar{x}$  + SD (n = 5) A single meristematic tip served as a replicate.

7 Total number of larvae (dead and alive) and pupae were used to compute % survival.

Table 5. Results of multiple choice oviposition test with <u>Bayeria capitigena</u> A Rome, Italy, 1982.

1taly, 1982.			
Test Plants	No.of exposed apical tips	% of apical tips with eggs	% of apical tips with galls
EUPHORBIACEAE			
Euphorbia esula L. (Pisa, Italy)-Control	31.00 <u>+</u> 19.30	43.10+20.95	39.67+14.84
E. esula-virgata "complex" Nebraska type	12.00 <u>+</u> 1.73	61.80 <u>+</u> 20.47	50.76+17.31
E. esula-virgata "complex" Montana type	11.00 <u>+</u> 2.64	67.00 <u>+</u> 2.56	63.30+ 7.25
E. esula-virgata "complex" Oregon type	9.33+ 0.58	27.23 <u>+</u> 11.60	27.23+11.60
E. esula-virgata "complex" Saskatchewan type	6.33 <u>+</u> 1.53	53.70 <u>+</u> 11.60	50.36+14.31
E. cyparissias L.	25.00 <u>+</u> 8.90	33.10 <u>+</u> 14.81	30.63 <u>+</u> 12.51
E. lathyris L. "Castro Valley"variety CA.	2.00+ 0.00	100.00 <u>+</u> 0.00	100.00 <u>+</u> 0.00
E. lathyris L. "Chico" variety CA.	2.00+ 0.00	100.00 <u>+</u> 0.00	100.00 <u>+</u> 0.00
E. peplus L. (Palermo, Italy)	36.67 <u>+</u> 9.07	29.80 <u>+</u> 6.07	26.57 <u>+</u> 4.73
E. characias L. (Trieste, Italy)	11.67 <u>+</u> 3.05	17.90+ 4.46	-
E. myrsinites L. (Trieste, Italy)	8.67 <u>+</u> 2.90	18.67 <u>+</u> 21.19	13.10+12.54
E. serrulata Thuill. (Nantes, France)	22.67+ 3.21	9.06 <u>+</u> 8.44	9.06+ 8.44
E. marginata Pursh.	3.67 <u>+</u> 1.15	-	-
E. pulcherrima Willd. ex Kl.	13.33 <u>+</u> 4.04	em '	es.
E. heterophylla Linn. Amoeana	10.33+2.08	-	-
E. milii Ch. des Moulins	40.67 <u>+</u> 2.52	-	· —
E. tirucalli L.	9.00+ 3.46	-	-
Ricinus communis L.	6.00+ 1.73		
Codiaeum variegatum(L.) Blume var.pictum(Lodd).	3.00 <u>+</u> 1.00	-	-
RUTACEAE			
Ruta graveolens L.	10.00 <u>+</u> 2.65	-	-
LINACEAE			
Linum narbonense L.	69.00+14.22	-	-

# GERANIACEAE

Geranium rotundifolium L.	15.00 <u>+</u> 1.00	-
Pelargonium zonale (L.) L'Her.	10.33+ 3.52	-
ROSACEAE		
Rosa sp.	8.00 <u>+</u> 2.65	-
UMBELLIFERAE		
Daucus carota subsp.sativus (Hoffm.)	8.00 <u>+</u> 1.00	_
FABACEAE		
Phaseolus vulgaris L.	11.00 <u>+</u> 1.00	-
Medicago sativa L.	155.33+47.09	-
ONAGRACEAE		
Clarkia elegans Dougl.	9.00 <u>+</u> 2.65	
APOCYNACEAE		
Nerium oleander L.	13.00 <u>+</u> 1.00	-
Vinca major L.		
ASCLEPIADACEAE	•	
Asclepias syriaca L.	3.33 <u>+</u> 1.53	-
Asclepias curassavica L.	2.33+ 0.58	_
SOLANACEAE		
Solanum tuberosum L.	13.67 <u>+</u> 7.51	
CONVOLVULACEAE		
Ipomoea batatas (L.) Lam-cv. Garnet	26.00 <u>+</u> 1.00	-
LABIATAE		
Salvia splendens Ker. Gavl.	15.00 <u>+</u> 5.20	-
Thymus serpyllum L.	136.67 <u>+</u> 34.03	-
SCROPHULARIACEAE	,	
Antirrhinum majus L.	21.67 <u>+</u> 3.05	

# COMPOSITAE

Lactuca sativa L. cv. Great Lakes	4.00 <u>+</u> 2.00	-	-
Cichorium intybus L.	11.00 <u>+</u> 5.29	- '	-
Carthamus tinctorius L. cv. Dart	8.33 <u>+</u> 3.51	-	-
Helianthus tuberosus L.	5.00 <u>+</u> 1.73	-	_
GRAMINEAE			
Zea mays L Field corn (B73 x M017)	2.00 <u>+</u> 0.00	-	-
Zea mays L Sweed corn-(Golden hybrid blend)	2.00 <u>+</u> 0.00	-	-
BRASSICACEAE			
Brassica oleracea L. Capitata Group	4.00 <u>+</u> 0.00	-	-
MORACEAE .			
Ficus elastica Roxb. ex Hornem	4.00 <u>+</u> 0.00	-	_
CHENOPODIACEAE			
Beta vulgaris L. cv. Early wonder	4.00 <u>+</u> 0.00	-	-
RANUNCOLACEAE		,	
Anemone sp.	12.00+ 3.00	-	-
	•		

 $<sup>\</sup>underline{a}/\overline{X} + SD \quad (n = 3)$ 

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# 2) Oncochila simplex H.-S. (Heteroptera: Tingidae)

Screened by USDA Rome Laboratory. The insect had been introduced in quarantine into US. In laboratory tests at Albany, Ca.,  $\underline{0}$ . simplex eggs were found on both lettuce and corn. Randomized open field tests were therefore made at the Rome lab with 2 varieties of lettuce and 2 varieties of corn. A few  $\underline{0}$ . simplex eggs were found on these crops. However, no new adults emerged from the lettuce or corn plants. Instead, a new generation was obtained on the "control" plant,  $\underline{E}$ .  $\underline{e}$ sula. We hope, therefore, to obtain the permission to release the insect in the  $\underline{U}$ . A petition for its introduction will be submitted soon.

# 3) Oberea erythrocephala Schrank (Col: Cerambycidae)

Screened by CIBC. Released in Canada and the U.S. Appears to be difficult to establish. To supplement previous releases, a large collection was made at San

Rossore, Pisa and shipped to Albany, California.

The <u>Oberea</u> adults had been collected on <u>Euphorbia esula</u> (n = 1,212) and on <u>E. cyparissias</u> (n = 636). During the entire month of June a person was stationed in the area for the collection. It is very difficult to find large numbers of this insect anywhere in Europe. The San Rossore site, in Italy, is an exception. We were lucky to find relatively large areas of <u>E. esula</u> with a good <u>Oberea</u> population. We should avoid, anyhow, frequent and <u>massive</u> collections there, since we could possibly destroy the area as a collection site for many years.

# 4) Chamaesphecia tenthrediniformis Den. and Schiff. (Lep: Aegeriidae)

The species seems to be restricted to Euphorbia esula (s. str.) and does not

accept North American "ecotypes" of leafy spurge.

A trip was made in eastern Europe from Oct. 21 to Nov. 12, 1982 to locate possible Chamaesphecia strains on E. virgata. Locations in Austria, Hungary and Rumania were visited. However, no Chamaesphecia larvae were found on E. virgata even when both species, E. esula and E. virgata were intermixed in the same site. In some of these sites we have found the 90% of E. esula roots infested by Chamaesphecia larvae. During the trip 500 E. esula roots infested by C. tenthrediniformis larvae were collected in Hungary. These roots will be shipped on March 1983 to Albany quarantine for release on E. esula s. st. in the U.S.

# 5) Aphthona flava Guill. (Col: Chrysomelidae)

Aphthona flava as well as A. cyparissiae (Koch) were screened by CIBC. Both beetles have been cleared for release in Canada.

As per request, 2,000 adults were collected on <u>Euphorbia esula</u> at San Rossore, Pisa on June 1982 and shipped to Peter Harris for direct release in Canada.

# 6) <u>Lobesia euphorbiana</u> Frr. (Lep: Tortricidae)

Screened by Peter Harris, Canada. Final report submitted for clearance. As per request, 105 infested tips of  $\underline{\text{Euphorbia}}$   $\underline{\text{lucida}}$  and 55 of  $\underline{\text{E.}}$   $\underline{\text{cyparissias}}$  were sent to Peter Harris to renew his  $\underline{\text{Lobesia}}$  colony.

# 7) <u>Neoplinthus tigratus</u> Rossi (Col: Curculionidae)

A root borer. Damage severe. Larval host <u>Euphorbia esula</u>. Adult food source unknown. Little work had been done on this insect. More emphasis will be given on the coming season.

# 8) Simyra dentinosa F. (Lep: Noctuidae)

Leaf feeder. Cause severe plant defoliation. Found in Afghanistan (Dunn) and Greece (Rizza and Pecora) on <u>Euphorbia seguieriana</u>. No work was done at Rome on this insect during 1982.

# 9) Dasineura capsulae Kieff. (Dipt: Cecidomyiidae)

A univoltine flower and fruit gall midge. Our preliminary studies indicate that this species is quite difficult to handle. In conducting preliminary host specificity tests it was very difficult to have a perfect synchrony of the occurrence of the adults and flowers of test plants. The species is of low priority, as biocontrol agent.

Euphorbia spp.//

R. Sobhian, Thessaloniki, Greece

# Euphorbia spp.

It was decided to send Euphorbia esula L. (Complex) plants from Rome to Greece and test them for acceptability by Simyra dentinosa Freyer, a noctuid found on

Euphorbia seguierana Necker in Greece.

On April 30, 3 egg masses of  $\underline{S}$ . dentinosa were collected from  $\underline{E}$ . seguierana at a site about 60 km east of Thessaloniki for a first instar larval test on the  $\underline{E}$ . esula plants. It was noticed that an egg predator had eaten some eggs in 2 of the egg masses. The test for which the eggs were collected could not be completed because test plants did not arrive from Rome.

Specimens of E. seguierana infected with a rust disease were prepared and mailed to Drs. Bruckhart and Defago. Dr. Defago was interested because she

wanted to study the overwintering pattern of the rust.

'On May 21, about 150 <u>S. dentinosa</u> larvae were collected and reared to pupation; they are currently overwintering as pupae. The larvae pupated among the dried leaves of their food plants or on the cage walls, in a very dense cocoon. When examining the site with Dr. Pemberton, USDA Albany Laboratory, a pupa was found

near the ground among the stems of the host plant.

Six samples of  $\underline{E}$ . esula seed were received from Albany; 4 of US and 2 of Canadian origin. These will be used to provide plants to conduct first instar feeding trials. They were planted in pots kept on an asphalt road so the roots could not reach the ground. In the fall 20 root samples arrived from Albany. These were also placed in pots on the asphalt surface and will be used for  $\underline{S}$ . dentinosa first instar larval feeding trials in 1983.

Musk Thistle (<u>Carduus "nutans"</u>)

A. Rizza, G. Campobasso

A syrphid fly with a phytophagous larva <u>Cheilosia grossa</u> (Fallen) was tested in both the laboratory and field and found to be a promising, highly

specific, very destructive natural enemy of musk thistle.

The fact that two of the 25 first instar larvae could survive on <u>Cirsium crassicaule</u> (Greene) Jeps., (a native and sometimes rare California <u>Cirsium spp.</u>) which was one of a series of 10 <u>Cirsium spp.</u> tested in this way, necessitated a host selection test using a naturally occurring C. grossa population.

The randomized block field trial design and technique, using North American Cirsium spp. and native musk thistle controls is a model for all future tests

where the essential elements are present.

The data supporting the introduction of this candidate for field release are presented in the accompanying petition for introduction.



A petition in support of the release of <u>Cheilosia grossa</u> (Fallen) (Diptera: Syrphidae), a candidate for the biological control of musk thistle, <u>Carduus nutans L.</u> species group in North America.

Prepared by:

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#### I. Introduction

Carduus nutans L. (Compositae, Cynareae, Carduinae), was probably introduced into the US from Europe. The weed, commonly called musk thistle, was first recorded in the US in 1853 at Harrisburg, Pennsylvania (Stuckey and Forsyth 1971). It has become an important weed in much of the United States, with the largest and most severe infestations in the Appalachian and midwest regions (Dunn 1976). Sixty-five thousand hectares in Virginia and Nebraska alone have been infested (Dunn 1976). When established in pastures the weed competes with desirable plants and limits the use of these areas by livestock or for recreational purposes because of the spines on the leaves, stalks, and blooms (Batra 1978).

The taxonomy of the musk thistle group is not clearly defined. The many different forms, generally referred to in the botanical literature as "Carduus nutans L.", were taxonomically arranged by Kazmi (1964). McCarty (1978), using Kazmi's revision of the Carduus group, and information from the Flora Europaea (Tutin et al 1976), believes that most Carduus thistles in the United States are C. thoermeri Weinmann and that some forms like C. nutans and C. macrocephalus

Desfontaines are also present.

The first attempt at biological control of musk thistle involved the weevil Rhinocyllus conicus (Froelich). This weevil oviposits on the bracts of thistle heads and larvae feed on the developing seeds. In 1969 this weevil was released in Montana (Rees 1977) and Virginia (Surles et al 1974); it is now well-established in several states in the US (Andres and Davis 1973).

The larvae of a second weevil, <u>Trichosirocalus horridus</u> (Panzer), damage thistle rosettes by feeding on the meristematic tissue; it was released in the US in 1974. Kok and Trumble (1979) confirmed initial establishment of the

weevil in Virginia.

We propose for introduction into the US a third insect, Cheilosia grossa (Fallen) (Diptera: Syrphidae). Larvae of this fly attack the stems and roots of C. nutans and C. pycnocephalus L. This introduction is fully justified for the following reasons: 1) The insect is a highly effective natural enemy of its host plants; 2) It attacks a different part of its hosts and does not compete with R. conicus and T. horridus; 3) The combined action of several biological agents will result in more stress on targeted host plants, resulting in better biological control; 4) Differential timing of attack by several natural enemies will stress its hosts over a longer period of time; 5) Literature records together with field and laboratory tests indicate that the insect is host specific; 6) The insect can be also used as a biological control agent of Italian thistle, Carduus pycnocephalus, another weed of economic importance in the U.S. (Dunn 1976).

#### II. Taxonomic Position

<u>Cheilosia grossa</u> (Fallen) (Syrphidae, Milesiinae, Cheilosinii). The adult was described by Seguy (1961). Dusek and Laska (1962) described and illustrated

the larvae and puparia.

The genus <u>Cheilosia</u> Meigen contains about 130 Palearctic species (Smith 1979). Of these, the immature stages of only a few species have been described. Many larvae are phytophagous and feed on either fungi or in the stems of plants (often

Compositae).

A list of European Cheilosia species and their known hosts is given by Smith (1979) (Table 1). Other important Milesiinae are Merodon equestris F. and Emerus stringatus Fall., whose larvae feed on various bulbs, particularly Narcissus and Gladiolus (Della Beffa 1961).

#### III. Geographic Distribution

Cheilosia grossa is reported from Austria, Switzerland, Germany, Denmark, Sweden, Dalmatia, Hungary, Russia, Poland and Scandinavia (Becker 1894). Seguy (1961) gives its distribution as middle and northern Europe. Mellini (1951) reported its presence in Italy.

#### IV Host Plants

Cheilosia grossa has been recorded on various thistles. Nurse (1910) reported finding larvae of <u>C. grossa</u> in the basal portion of stems of <u>Cnicus palustris</u> (L.) Willd. (= <u>Cirsium palustre</u> (L.) Scop.) in August. Dusek and Laska (1962) found larvae in stems of <u>Carduus crispus</u> L.. Mellini (1951) reported that larvae overwinter in roots of <u>Carduus nutans</u> L. and <u>C. pycnocephalus</u> L., near Bologna, Italy, with adults emerging in March. <u>Cheilosia grossa larvae</u> have been collected by us in many locations in Italy (from sea level to 1300 m), but only in the stems and roots of <u>C. nutans</u> and <u>C. pycnocephalus</u>.

There are no literature records of <u>C. grossa</u> as a pest of cultivated plants (Balachowsky 1963, Della Beffa 1961, Review of Applied Entomology, series A.

1913-73).

# V Life History

Material and Methods: The seasonal sequence of the various  $\underline{C}$ .  $\underline{grossa}$  life stages was followed during 1980-82 at Castel Porziano, a government hunting preserve near Rome, Italy. This preserve of 5,000 hectares, mostly uncultivated, supports a good infestation of  $\underline{C}$ .  $\underline{nutans}$  and a high population of  $\underline{C}$ .

grossa.

This study began in July 1980 by field marking  $100 \ \underline{C}$ . nutans plants infested with  $\underline{C}$ . grossa larvae. This was necessary since the aerial portion of dead  $\underline{C}$ . nutans disappear during the winter. In late January 1981,  $\underline{C}$ . grossa pupae (n=21) were collected from the roots of dead  $\underline{C}$ . nutans plants previously marked. These pupae were placed in soil at Castel Porziano at a depth of 8-10 cm. A square wooden frame cage (50 x 50 x 50 cm), covered with nylon screening, was placed over the ground where the pupae were placed to enable us to collect the emerging flies.

The oviposition period was followed during the 1981 and 1982 flight seasons. One hundred randomly selected  $\underline{C}$ . nutans plants were inspected every other day for eggs. Those eggs discovered were recorded, then crushed. With this technique it was possible to record the oviposition cycle, peak oviposition,

average number of eggs per plant, and % of plants infested.

Periodical dissections of  $\underline{C}$ .  $\underline{nutans}$  plants (n = 20) were made in the field during 1981 to follow the sequence of larval development and the pupation

period.

Larval instar development was studied in an insectary under natural conditions of temperature and light. Fifteen neonate larvae (one/cup) were put on shoots of <u>C. nutans</u> plants, held in 175 cc plastic cups. Each shoot was dissected on Monday, Wednesday and Friday to record larval moulting. Each dissected shoot was replaced with a fresh one and the larva was placed inside the new shoot. When the larvae reached the third instar, the food was replaced once a week until pupation.

Results: The adults all emerged by March 2 in the cage. This indicated that the flies should be flying in the field. However, after 3 days of looking for the flies on C. nutans at Castel Porziano, we had no success. We did observe 2 adults flying in the field during this time. In the meantime, we carefully examined the 100 C. nutans plants for eggs (three times per week). In 1981, the first eggs (n =  $\overline{2}$ ) were found on March 10. Peak oviposition occurred around March 24 with a mean of 5.88+7.85 ( $\overline{x} + SD$ ) eggs per plant (range = 1-60). Sixty-four per cent of the  $1\overline{00}$  plants had eggs. The oviposition cycle ended on April 2. In 1982, the first eggs (n = 8) were found on March 26, with peak oviposition occurring around April 10; mean of 3.92+3.93 eggs per plant attacked (range = 1-20). Sixty percent of the 100 plants had eggs. The oviposition cycle ended on April 19.

The eggs are layed on the young hirsutulous leaves and young shoots and usually at the center of the plant where the leaves and young shoots are more compact. The tangled leaves and shoots probably protect the eggs against parasites and predators and from direct sun exposure, and guarantee enough moisture for development. Eggs are most frequently laid singly but can be found also in groups of 3 or 4. Under outdoor temperatures, eggs require 6.42±.69 days (mean of 85

eggs) to hatch.

There are 3 larval instars. In a laboratory experiment, larvae needed 11.20±0.56 days to reach the II instar and '6.66±1.34 days to reach the III instar. The larvae actively fed as III instars until November when they pupated.

In nature, newly hatched larvae mine directly into tender young shoots. As the shoots grow, II and III instar larvae penetrate the more compact tissue of stems, tunnelling toward the root of the plant. We often found more than one

larva per shoot or stem.

On one of the dates (April 29, 1981) when we randomly selected <u>C. nutans</u> plants at Castel Porziano for dissection, we found, for example, that 13 of 20 (65%)plants had <u>Cheilosia</u> larvae (3.69+4.48, range 1-29). The number of shoots per plant was 6.85+3.92; the mean number of infested shoots per plant was 3.08+3.80.

By late-April, III instar larvae were found actively feeding in the stem galleries. Feeding continued through the summer and into the fall during which time feeding by III instars gradually extended into the crown and root. Mature larvae were found in October. Pupation was completed by late November. Mature larvae generally pupated in a root, but some pupated in nearby soil (5-10 cm below the surface) when a root was completely destroyed.

#### VI Mortality Factors

A thousand eggs, collected intermittently during the 1981 and 1982 oviposition period, were held in small plastic hatching cups (50/cup), as described by Rizza (1977), to capture emerging parasites. No parasites emerged.

Field collected larvae were found parasitized by Phydogeuon sp. (Hymenoptera: Ichneumonidae). Percent parasitization has not been determined.

#### VII Effect on Host Plants

The full value of the feeding damage to the host plant is clearly evident. The Cheilosia attack, besides stressing the plant, severely reduces seed production by destroying the shoots. Each shoot produces more than one flower

head. When more than one larva reaches the root, this often results in the death of the plant.

More details in Section V.

#### VIII Potential Control Value

Effectiveness rating for C. grossa (P. Harris (1973) scoring system)

		Score
3. 4. 5. 6. 7. 8. 9.	Period of attack is prolonged Univoltine species Lays less than 500 eggs Subject to mortality from specialized enemies (% unknown) More larvae can be found in the same stem Good compatibility with other insect Covers full range of the target weed Controls hosts in native habitat	3 5 0 4 0 0 4 2 2 6 4 0
		30

# IX Host Specificity Experiments

A recent USDA recommendation for the screening of biological control agents of weeds calls for the mandatory inclusion of US endangered plant species closely related to the target weed in the testing protocol. This new requirement involves a series of new problems related to the manipulation of exotic plant species, sometimes weedy, that must be grown in the country where tests are to be made, in this case Italy. While the plants are grown only in pots and not allowed to flower, some element of risk of escape is always present, thus complicating the work of the overseas laboratory. This testing of Nearctic plant species in Italy is possible only because of the courtesy and cooperation of the Italian Ministry of Agriculture.

Reported here are the results of host specificity tests which were conducted to determine the host range of  $\underline{C}$ . grossa. The studies were conducted at the Biological Control of Weeds Laboratory, Rome, Italy from 1980 to 1982.

# Larval Starvation Test (Laboratory)

Material and Methods: To determine the range of plants that would support C. grossa development in the laboratory, field collected eggs were transferred to 35 plant species in 6 families (Table 2). The plants were selected because they were: a) closely related to the target weed; b) hosts of congeners of Cheilosia; c) crops in the same family as the target weed; d) endangered and native US plants closely related to the target weed.

The plants used in the test were transplanted from the field as young roset-

tes (the thistles), or reared in the lab greenhouse (the cultivated and the US Cirsium species). When used in the test, the plants were all big enough to support an eventual larval attack. The plants were all potted and during the test were maintained in an outdoor insectary. Five replicates were used for each plant species. Each plant was infested with 5 mature eggs. The eggs (over 1,000) were collected at Castel Porziano, near Rome, from April 2 to 5, 1982. The chorion of all the eggs used was recovered to make sure they had hatched.

The test started on April 6, 1982, and ended two months later when all the plants were dissected.

Results: Very little is known about female ovipositional behavior. According to Smith (1979) Cheilosia adults often frequent flowers of the same species of plants in which their larvae develop. In the case of C. grossa, the known hosts C. nutans and C. pycnocephalus are not in bloom during the oviposition period; they are generally in the rosette stage. A chemical cue(s) emanating from the host plant may serve as the host recognition and oviposition stimuli, or some other host cue may trigger this behavior. Without knowledge of the mechanism for mating, host selection, and oviposition, it is difficult to design laboratory experiments to determine oviposition preference. Consequently our laboratory host specificity tests are based on a "theoretical oviposition", that is, placing the eggs on the test plants. We preferred to use eggs instead of neonate larvae to avoid stress during transfer.

The results (Table 2) clearly show that the host range of C. grossa is very narrow, involving only plants in the tribe Cardueae. Carduus nutans and C. pycnocephalus are the known European hosts. Cirsium crassicaule is an extension of the range in the laboratory. The mean number of larvae in the C. nutans and C. pycnocephalus plants was 1.40+.55 and 1.60+.55, respectively. Only 2 of the 5 plants of  $\underline{C}$ . crassicaule had larvae (one larva/plant for a mean of Cirsium palustre, the third recorded host in the literature, was 0.40+0.55). This can be attributed to incorrect identification of not a suitable host. the plant or insect.

# Oviposition Preference (Field)

Material and Methods: Since Cheilosia oviposition behavior cannot be realistically assayed in the laboratory or insectary, two field tests were conducted to measure the oviposition behavior of flies when given a choice between their natural hosts and US Cirsium species.

In devising the testing scheme for this open field oviposition test, we wanted to increase the probability that the test plants would be visited by

ovipositing flies.

Because the flight range of the flies is not known, there was a major problem to solve: What distance should separate US Cirsium from the naturally growing musk thistle plants? Would the flies be able to find a US Cirsium plant if a great distance separated the plants? On the other hand, we could run the risk of having the natural host plants exert an over-riding influence on oviposition behavior if the US Cirsium were placed too close.

Test (a) was set-up to try and force the ovipositing Cheilosia on the various Cirsium species, putting them nearby the naturally growing musk thistle. With Test (b), by finding eggs on the natural host (musk thistle) we were sure that the area had been visited by the flies; then, by removing the natural host, and leaving only the test plants, we avoided any influence of the natural

musk thistle on oviposition.

Test (a): A plot of 500  $m^2$  (20 x 25 m) was chosen at Castel Porziano in an area with a C. grossa population. All naturally growing musk thistle plants in the plot were numbered and mapped out (n = 115 in total) (Fig. 1). From these plants, 24 were randomly selected to be "attraction plants". Four potted plants (= replicates) of each of the US Cirsium species (6 species available) were used. Test plants and controls, grown from seeds in the green-

house, were potted and buried to ground level.

The rosette diameter of "attraction plants" (A.P.), "controls" (C.), and "test plants" (T.P.) were measured at the beginning of the experiment (March

29, 1982).

To each "A.P." (natural <u>C. nutans</u> left in place) was randomly assigned a "T.P." (potted <u>U.S. Cirsium</u>) and a "C." (potted <u>C. nutans</u>). A distance of 30 cm separated the plants. Position of "T.P." and "C." in relation to "A.P." was by random placement in one of four positions (see diagram in Fig. 2). Every 2 days all plants were carefully examined for eggs. After recording the presence of eggs, they were removed, except on the last examination (April 13).
"T.P." and "C." were exposed to flies during the peak oviposition period (March 29-April 13). All "T.P." and "C." were removed from the field plot on April 13 and transferred to the weed garden, Rome Lab., where they were left undisturbed for 2 months. After this time they were dissected to check for larval survival.

Test (b): On March 29, a field of 288  $m^2$  (12 m x 24 m) was chosen in the same area of Test (a) (50 m distance). The field was divided into 32 plots of 9 m $^2$  each (3 m x 3 m) (Fig. 3). A "T.P." or a "C." plant was placed in the center of each plot. "T.P." and "C." were assigned by random to each plot. All the plants, grown from seeds in the greenhouse, were potted and buried to ground level. Four potted plants (= replicates) of each of the U.S. <u>Cirsium</u> species (7 species available) and of C. nutans (control) were used. The musk thistle plants, naturally growing in the plots, were mapped out and left in situ to ascertain that the area was visited by Cheilosia adults. On March 31, eggs were found on 8 of the 75 musk thistle plants existing in the plots. On April 2, an additional 15 musk thistle plants were found with C. grossa eggs. On this date all the naturally growing musk thistle plants were removed from the plots, leaving only the "T.P." and the "C.". "T.P." and "C." were exposed from March 29 to April 13. Rosette diameter of "T.P." and "C." were measured at the beginning of the test. Every 2 days, "T.P." and "C." were examined and eggs found were removed after recording. "T.P." and "C." were removed from the field plots on April 13, and transferred to the weed garden, Rome Lab, where they were left undisturbed for 2 months. After this time they were dissected to check for larval survival,

Results: The results are very encouraging. In Test (a) (Table 3), 91.7% of the "A.P." and the 95.8% of the "C." had eggs. No eggs were found on the 24 "T.P.". The "A.P." appeared to have no influence on oviposition since the "C." had eggs. The non oviposition on Cirsium plants cannot be ascribed to the rosette size of these plants. There were no statistical differences (T-test, P=0.001) between rosette diameters of "T.P." and their respective "C.". A statistical difference (T-test, P=0.01) existed in only 3 of the paired comparisons of mean rosette diameters (see Table 3). In addition, 2 months later, 91.7% of the "C." had Cheilosia larvae (mean of 1.64+.66 larvae/plant). Larvae were not found in the "T.P.".

In Test (b), as well, none of the "T.P." served as suitable oviposition sites for Cheilosia. In contrast, all of the "C." had eggs. The rosette diameters of the various "T.P." were not significantly different (T-test, P=0.01) from those of the "C.". The area was visited by ovipositing flies since eggs were found on the randomly distributed "C." When all the plants were dissected 2 months later, only the "C." were found with larvae (mean of 2.75+.50 per plant).

# Dissection of Weedy Thistles (Field)

Material and Methods: To determine the host range of <u>Cheilosia</u> in nature, various thistle species were randomly selected (see Table 5 for a list of species) in June 1981 from Castel Porziano, Rome (sea level) and Sila, Calabria (1,300 m altitude) and dissected. <u>Cheilosia grossa</u> is common in both areas. Fifty and 100 plants per thistle species were dissected from Castel Porziano and Sila respectively. All plants were dissected in <u>situ</u> and the larvae found were kept for identification. Herbarium sheets were made of the thistles from both sites for identification purposes.

Results: Table 5 shows the results of the dissections. In both sites, larvae were found only on the known hosts  $\underline{C}$ .  $\underline{nutans}$  and  $\underline{C}$ .  $\underline{pycnocephalus}$ . At Castel Porziano, 70% of  $\underline{C}$ .  $\underline{nutans}$  and 36% of  $\underline{C}$ .  $\underline{pycnocephalus}$  plants were attacked by  $\underline{C}$ .  $\underline{grossa}$  (mean larvae 1.71+.86 for  $\underline{C}$ .  $\underline{nutans}$  and 2.44+1.89 for  $\underline{C}$ .  $\underline{pycnocephalus}$ ). In Sila, 41% of  $\underline{C}$ .  $\underline{nutans}$  plants were attacked by  $\underline{C}$ .  $\underline{grossa}$  (1.72+0.79 larvae/plant).  $\underline{Carduus}$   $\underline{pycnocephalus}$  and other thistles were not present in Sila. The larvae found on  $\underline{Cirsium}$  spinosissimum (L.) Scop. and  $\underline{Cirsium}$  sp. have been identified as  $\underline{Cheilosia}$   $\underline{variabilis}$  Panzer by Dr. J. P. Lyon, a French syrphid specialist.

#### X Discussion

The most important components of host choice are: habitat preference, ovipositional preference and larval feeding preference. An interaction of these components determines the host range of phytophagous insects. For instance, suitable hosts for larval development may be excluded from the host range due

to their occurrence in the wrong habitat.

The possession of wrong ovipositional stimuli or the absence of a precise recognition feature are also limiting factors for host choice. The fact that Cirsium crassicaule was found to be a marginally suitable host (2 of 25 larvae survived) of C. grossa in a manipulated laboratory test, does not necessarily mean that C. crassicaule contains the necessary recognition features or grows in the right habitat to be attacked in the field. Indeed, in a field oviposition experiment, using replicated and randomized plantings of US Cirsium species, there was no oviposition on any of the Cirsium. This experiment was conducted in an area supporting a high C. grossa population.

in an area supporting a high <u>C</u>. <u>grossa</u> population.

According to the California Native Plant Society Rare Plant Status Report (Anonymous 1979), <u>C</u>. <u>crassicaule</u> is under review for threatened species status. This annual or biennial plant grows, "usually on banks of streams, washes, slough, or canals; sometime in moist to wet places". This is not the right habitat in Europe for <u>C</u>. <u>nutans</u> and <u>C</u>. <u>pycnocephalus</u>, which prefer well drained soil (Fiori 1974, Tutin <u>et al</u>. 1972). Moreover, it is possible that in the United States populations of <u>C</u>. <u>crassicaule</u> and musk thistle could overlap, but this should be confirmed. In any case, our field experiment showed that <u>C</u>. <u>crassicaule</u>, as well as the other <u>Cirsium</u> species tested, lack the necessary recognition features for <u>C</u>. grossa oviposition.

recognition features for <u>C. grossa</u> oviposition.

It is also questionable if <u>C. crassicaule</u> is a threatened species. The aforementioned Report states that <u>C. crassicaule</u> "appears to be a common canal bank weed", and that in 1979 a new population of this plant containing "thousands

of plants along the Kern River at 10th Avenue, Corcoran, Kings Co." was found. In conclusion, field observations and host specificity tests conducted in the field, together with literature records, indicate that <u>C. grossa</u> is host specific and, consequently, safe to introduce into the US for the biological control of musk thistle.

Because the fly has been found to be common throughout the range of climates where <u>Carduus</u> spp. are found in the United States, there should be no difficulty

in establishing it in the new environment.

#### XI. Summary

Musk thistle, <u>Carduus nutans</u> L. (Compositae, Cynareae, Carduinae) is an important weed in much of the United States, with the most severe infestations

in the Appalachian and midwest regions.

Previous attempts at biological control of musk thistle involved the thistle-head weevil Rhinocyllus conicus (Froelich), the larvae of which feed on the developing seeds, and Trichosirocalus horridus (Panzer) the larva of which damages thistle rosettes by feeding on the meristematic tissue. Both weevils are now established into US.

We propose for introduction into U.S. a third insect, <u>Cheilosia grossa</u> (Fallen), a syrphid fly widely distributed in Europe. Larvae of this fly attack the stems

and roots of Carduus nutans and C. pycnocephalus L.

Our laboratory tests, confirming literature records, show that the host range

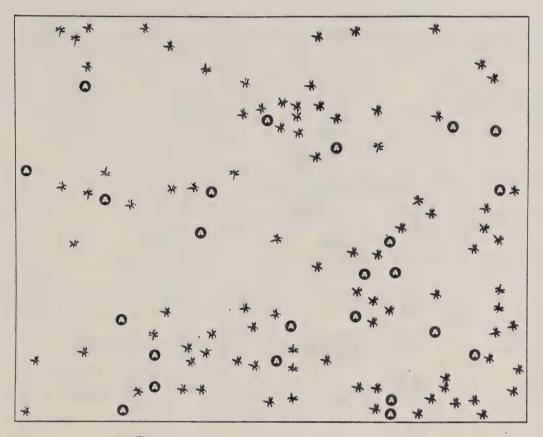
of C. grossa is very narrow, involving only plants in the tribe Cardueae.

Cirsium crassicaule, a native U.S. species, was found to be suitable marginally as a larval host in the laboratory. However, in field oviposition tests, using replicated and randomized plantings of US <u>Cirsium</u> species (including <u>C. crassicaule</u>), no oviposition was observed on any of these <u>Cirsium</u>. We believe, therefore, that <u>C. grossa</u> is host specific and safe to introduce into the U.S. for the biological control of musk thistle.

The introduction is fully justified for the following reasons: 1) The insect is a highly effective natural enemy of its host plants (effectiveness rating = 30); 2) It attacks a different part of its hosts and does not compete with R. conicus and T. horridus; 3) The combined action of several biological agents will result in more stress on targeted host plants, resulting in better biological control; 4) Differential timing of attack by several natural enemies will stress its hosts over a longer period of time; 5) Literature records together with field and laboratory tests indicate that the insect is host specific; 6) The insect can be also used as a biological control agent of Italian thistle, Carduus pycnocephalus, another weed of economic importance.

# XII. Figures and Tables

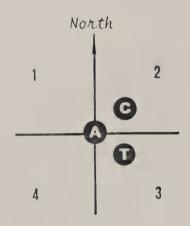
Fig. 1. Field plot of 500  $m^2$  (20 x 25 m) used for Test (a).



🧱 - Mush thistee plants growing in the field plot.

O . Musk thister plants randomly selected as "attraction plants

Fig. 2. Position of "Test plant" and "Control plant" in relation to "Attraction plant". Assigned by random to one of 4 positions, as pictured.



- A = Attraction plant (naturally growing C. nutans)
- **G** = Control plant (Potted <u>C</u>. <u>nutans</u>)
- Test plant (potted U.S. Cirsium)

Fig. 3. Field of 288  $m^2$  (12 x 24 m) divided in 32 plots of 9  $m^2$  each (3 x 3 m). Test (b).

	1	2	3	4	5	6	7	8
a	% <b>3</b>	3		<b>6</b>	**************************************	<b>(3)</b>	<ul><li>⊕</li><li>⊕</li><li>⊕</li><li>⊕</li></ul>	
b	- 1967 - 1967		@				in the second	<b>③</b>
С	0	4	<b>9 3</b>	AP (49)	Ap 48		\$\$ \$\$	<b>→</b>
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Musk thistle plants.

<sup>=</sup> Musk thistle plants sound with Cheilesia grossa eggs.

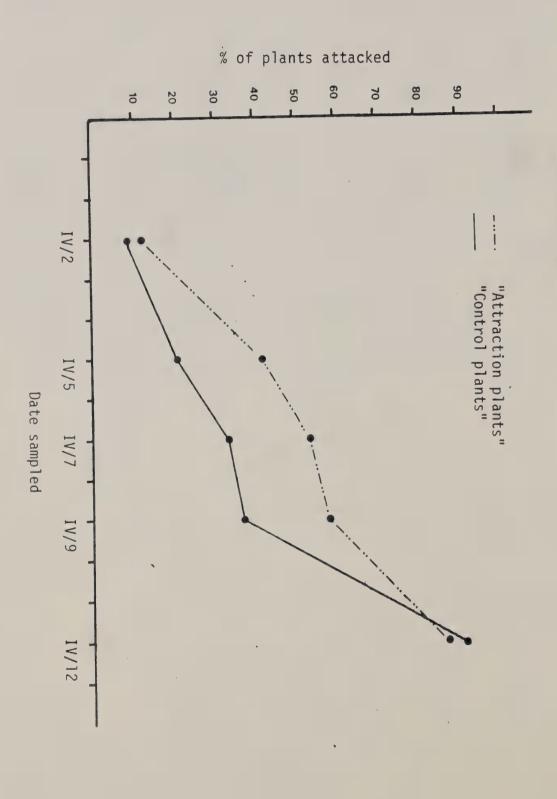


Fig. 4. Curves depict the % of "Attraction plants" and "Control plants" found with Cheilosia grossa eggs versus time, open field oviposition Test (a), Castel Porziano, 1982.

Table 1. The known biology of the European species of the genus <u>Cheilosia</u>, as given by Smith (1979).

CHEILOSIA SPECIES	PLANT (orig. nomenclature)	SOURCE
albipila (as chrysocoma)	Carduus crispus (stem-root)	Weyenburgh (1869)
alhipila	Carduus crispus, Cirsium	Boie (1850)
(as flavicornis) albipila	oleraceum (stem)	4 - 1 - (1044)
antiqua	Cnicus palustris (stems)	Andrews (1944)
(as sparsa)	Primula spp. (roots)	Carpenter (1913)
hergenstammi	Senecio jacobaca (roots, crowns)	present paper
canicularis	Petasites hybridus, albus, kablikianus (rhizome)	Dusek (1962)
ch!oris	Petasites niveus (roots)	Kaltenbach (1874)
cynocephala	Carduus nutans (stem)	Frauenfeld (1866) Dusck & Laska (1962)
fasciata	Allium ursinum (leaf-mine)	Beling (1888) Dusck & Laska (1962)
grossa	Cnicus palustris (stem)	Nurse (1910a, b)
grossa	Carduus crispus (stem)	Dusek & Laska (1962)
hercyniae	Amanita muscaria	Vimmer (1925)
longula	Boletus luridus, bovinus	Buxton (1955)
longula	Suillus & Leccinum	Hackman & Meinander (1979)
maculat <b>a</b>	Associated with Allium	needs further investigation
morio	'pine' (bark wounds)	Trägardh (1939)
mutabilis	Cardius acanthoides (root)	Rossi (1848)
nitidula	Matricaria chamomilla (stem)	Kaltenbach (1864)
omissa	Senecio nemorensis ssp. fuchsi	i Dusek (1962)
scutellata	rotten fungi	Roser (1834)
cutellata	Boletus edulis, pinetorum	Dufour (1840)
scutellata	Polyporus	Frauenfeld (1868)
scutellata	truffles	Goureau (1852),
(as & nr.	TT CONTROL	corrected
mutabilis)		Verrall (1901)
cutellata	Boleuts, Leccinum, Suillus	Eisfelder (1956)
scurellara	Boletus, Leccinum, Gyroporus,	Dely-Drascovits (1972)
	X crocomus	1703 1711111001113 (1772)
scutellata	Boletus, Pholiota	Chandler (1969)
scutellata	Leccinum	Hackman & Meinander
		(1979)
soror	truffles	Goureau (1852)
(as 9 nr.		corrected .
(cutellara)		Verrall 1901)
variabilis	Carduus nutans, acanthoides,	Kaltenbach (1874)
• • •	Cirsium lanceolatum (buds-stalks)	,,,,,
variabilis ·	Scrophularia nodosa (toots)	Fryer (1915)
variabilis	Scrophularia nodosa (100ts)	Dusek (1962)
velutina	Scrophularia nodosa (roots)	Brischke (1880)
(as gigantea)	,	,
vernalis?	Under decaying leaves	Dufour (1848) /
(aerea)	of Verbascum pulverulentum	/
vernalis?	Matricaria chamomilla	.Kaltenbach (1864)
(nitidula)	(stem root)	
Sp.	Truffles	Laboulbene (1864)
sp.	Truffles	Reaumer (1740)
sp.	Turnips .	Lunbeck (1916)
sp.	Onions	present paper

Family	Tribe	Test Plants		Replications				
			1	2	3	4	5	
Compositae	Cardueae	(h) Carduus nutans L.	1	1	2	1	2 2	
11	H H	(h) C. pycnocephalus L.	2	1	1	2	2	
11	··	(a) C. acanthoides L.						
11	tt.	(c) Cynara scolymus L.						
11	н	(c) <u>Carthamus tinctorius</u> L. (a) <u>Cirsium arvense</u> (L.) <u>Scop</u> .						
11	П	(a) <u>Cirsium arvense</u> (L.) Scop. (a) <u>C. vulgare</u> (Savi) Ten.						
14	H	(h) C. palustre (L.) Scop.						
11	H	(e) C. andrewsii (Gray) Jeps						
11	11	(e) C. discolor (Muhl.) Spreng						
11	11	(e) C. occidentale (Nutt.) Jeps						
11	Ш	(e) C. crassicaule (Greene) Jeps			1	1		
11	H .	(e) C. douglasii DC.						
11	II II	(e) C. foliosum (Hook) DC.		•				
11	"	(e) C. flodmani (Rydb.) Arthur						
п		(a) Silybum marianum (L.) Gaertner						
11	11	(a) Centaurea solstitialis L.						
ti e	н	(c) <u>C</u> . <u>cyanus</u> <u>L</u> . (a) <u>C</u> . <u>montana</u> <u>L</u> .						
11	н	(a) Cnicus benedictus L.						
11	9	(a) Galactites tomentosa Moench						
ŧŧ	Cichorieae	(c) Cichorium intybus L.						
	11	(c) Lactuca sativa L.						
11	Heliantheae	(c) Helianthus annuus L.					1	
"	Helenieae	(c) Tagetes erecta L.		•				
11	Calenduleae	(c) <u>Calendula officinalis</u> L.						
ů	Astereae	(c) Aster sp. L.					,	
)lantaginaceae	Anthemideae	(c) Chrysanthemum leucanthemum L.						
Scrophulariaceae	•	(b) Plantago lanceolata L.						
Primulaceae		(b) Verbascum pulverulentum Vill.						
\maryllidaceae		(b) Primula veris L. (b) Allium cepa L.						
11		(b) A. ursinum L.						
П		(b) Narcissus sp. L.						
Iridaceae		(b) Gladiolus communis L.						

weed closely related to the target (a)

<sup>(</sup>b)

host of other <u>Cheilosia</u> spp. crop in the same family as the target weed (c)

<sup>(</sup>h) known host

U.S. endangered species (e)

Table 3. Cheilosia grossa open field oviposition (Test a.).

Test Plant		Plant Diameter  X+SD1/ (range)	
	A.P. <sup>2</sup> /	c. <u>2</u> /	T.P. <u>2</u> /
<u>Cirsium</u> <u>discolor</u>	47.25 <u>+</u> 29.45	34.25 <u>+</u> 8.42	30.75+3.30 NS
	(16 <del>-</del> 83)	(26 <del>-</del> 42)	(26 - 33)
Cirsium occidentale	68.25+13.87 <sup>a</sup>	31.00+2.58 <sup>b</sup>	28.50+1.73 <sup>b</sup>
	(50 - 80)	(28 - 34)	(26 - 30)
Cirsium douglasii	43.75 <u>+</u> 11.93	35.25+4.27	26.25+4.50 NS
	(32 <del>-</del> 55)	(30 - 40)	(20 - 30)
<u>Cirsium</u> <u>foliosum</u>	53.50+12.77 <sup>a</sup>	32.75+7.14 <sup>ab</sup>	20.75+1.71 <sup>b</sup>
	(43 - 72)	(25 - 41)	(19 - 23)
<u>Cirsium craussicaule</u>	39.00 <u>+</u> 10.00	34.25+2.06	33.50+2.38 NS
	(26 <del>-</del> 50)	(32 - 36)	(30 - 35)
<u>Cirsium andrewsii</u>	46.75 <u>+</u> 17.54	35.25+2.99	33.25+6.18 NS
	(30 <del>-</del> 68)	(32 - 39)	(25 - 38)

<sup>1</sup>/ Means of 4 replications.

Means in a single row followed by the same letter are not significantly different by T test (P = 0.01)

<sup>2/</sup> A.P. = Attraction plant; C. = Control plant; T.P. = Test plant.

NS = No significant differences between mean values, T test (P = 0.01)

Table 4. Cheilosia grossa open field oviposition (Test b).

Test Plants	Reps	Randomly assigned plot		Plants found with eggs %	No.larvae recovered VI/14/82
Carduus nutans (control)	1 2 3 4	C 5 <u>2/</u> B 7 D 1 A 1	24.75 <u>+</u> 2.90 (21 - 28)	100	3 2 3 3
Cirsium discolor	1 2 3 4	D 8 B 3 D 5 A 8	31.50 <u>+</u> 2.65 (28 - 34)	Ø	
<u>Cirsium occidentale</u>	1 2 3 4	D 6 A 4 B 2 B 5	27.25+3.59 (22 - 30)	Ø	
<u>Cirsium</u> <u>douglasii</u>	1 · 2 3 · 4	B 1 B 6 B 4 C 7	25.00+5.10 (20 - 32)	Ø	
<u>Cirsium</u> <u>foliosum</u>	1 2 3 4	B 8 A 7 C 4 D 4	19.00±1.41 (18 - 21)	Ø .	1
Cirsium crassicaule	1 2 3 4	A 6 C 8 C 6 D 7	37.50+4.20 (32 - 42)	Ø	
Cirsium andrewsii	1 2 3 4	A 5 C 1 D 3 D 2	35.25+4.43 (31 - 40)	Ø	
Cirsium flodmanii	1 2 3 4.	A 2 C 2 C 3 A 3	19.75+1.50 (18 - 21)	Ø	

<sup>1/</sup> Mean of 4 replications

<sup>2/</sup> These plot combinations are shown on the grid depicted in Fig. 3. Means not significantly different by T test (P = 0.01)

Table 5. Results of di-sections of various thistle species in fields where Cheilosia grossa was present.

Plant	No.	Plant infested %	No. larvae/plant $(\bar{x} + SD)$	Larvae/plant (Range)
	Caste	l Porziano, Rom	ne	
Carduus nutans L.	50	701/	1.71 <u>+</u> .86	1-4
Carduus pycnocephalus L.	50	361/	2.44 <u>+</u> 1.89	7 7
Galactites tomentosa Moench	50			
Onopordum acanthium L.	50			
Silybum marianum (L.)Gaertner	. 50			
Cirsium arvense (L.) Scop.	50			
Cirsium vulgare (Savi) Ten. (=C. lanceolatum (L.)Scop)	.50			
Cirsium eriophorum (L.) Scop.	<del>5</del> 0			
Sonchus oleraceus L.	50			
Echinops sp. L.	50		• ,	
<u>Carlina</u> <u>acaulis</u> L.	50			
	Sila,	Calabria		
Carduus nutans L.	100	411/	1.72 <u>+</u> .79	1-4
Cirsium vulgare (Savi) Ten.	100			
Cirsium arvense (L.) Scop.	100			
Cirsium spinosissimum (L.)Scop.	100	13 <sup>2</sup> /	1.46 <u>+</u> .66	1-3
Cirsium_sp. Miller	100	<u>9</u> 2/	1.78 <u>+</u> 1.64	1-6
Onopordum acanthium L.	100			
Carlina acaulis L.	100			

<sup>1/</sup> Cheilosia grossa

<sup>2/</sup> Cheilosia variabilis

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Yellow starthistle and diffuse knapweed /

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# Yellow starthistle (YST)

The three important elements in this study in 1982 were: 1) The collection of the galls of the trypetid fly <u>Urophora siruna-seva</u> Hering on U.S. yellow starthistle varieties to be forwarded to the Albany Laboratory for release, as soon as the final data are in hand showing that they do not infest safflower.

Secondly, the work on the bionomics and biology of the weevil <u>Bangasternus</u> orientalis Capiomont (Coleoptera: Curculionidae) in Greece and its subsequent testing on a sufficient variety and number of plants in Rome showed that it was

specific enough to write a petition for introduction into quarantine.

Thirdly, an exploration was made in Turkey to find and precisely mark infestations of YST plants that could be visited in the spring of 1983 to search for natural enemies that attack the rosette and pre-flowering stage of the plant.

The results of the investigations in Greece on <u>U. siruna-seva</u>, <u>Bangasternus orientalis</u> and <u>Bruchidius tuberculatus</u> Hochh. (Coleoptera: Bruchidae) (which was subsequently disqualified as a candidate) plus other work is presented in more detail in the Attached Annual Report of the work in Greece. (Appendix I).

The work done on <u>Bangasternus orientalis</u> in Rome is presented in the form of a "petition for the introduction" of this insect into quarantine, which was prepared after the end of the calendar year, and contains the meaningful research

results. (Appendix II).

The project involving the exploration in Turkey is only half completed. Yellow starthistle was collected from 22 sites in that part of Turkey west of a line drawn from Samsun on the Black Sea, south to Adana on the Agean sea. Ten of these sites could merit another visit but are only six sites, widely distributed over western Turkey that will receive serious attention during the visit next spring.

#### <u>Diffuse knapweed - Centaurea diffusa Lam.</u>

The work on diffuse knapweed was pretty much concentrated on one insect

Pterolonche inspersa Stgr. (Lepidoptera: Pterolonchidae).

A petition to introduce <u>Pterolonche</u> into quarantine at Albany, California was written and accepted. This petition was based on the 1981 testing data which included oviposition choice tests on 5 plants in both large and small cages and a first instar larval survival trial on 12 plants. These data are summed-up in the copy of the petition included in this section. (Appendix III).

In addition to the submission and acceptance of the petition for the introduction of <u>Pterolonche</u> into quarantine, the major part of the host specificity testing necessary to prepare the petition for release of P. inspersa in the

United States and Canada was completed.

Forty nine test plants were prepared for this trial but some were early blooming annuals and had completely senesced by July, thus were so completely out of synchronization with the life cycle of  $\underline{P}$ .  $\underline{inspersa}$  that they could not be tested.

The data gathered from these tests are included in this section of the

annual report. (Appendix IV).



Appendix I
Centaurea spp.

R. Sobhian, Thessaloniki, Greece

#### Phenology of <u>Centaurea solstitialis</u> L. varieties

Seeds of yellow starthistle from 6 different locations in the United States (3 from California and 3 from Washington state) were germinated out of doors in

wooden boxes in October 1981).

In November 7 seedlings of each variety were planted in the garden of the Plant Protection Institute at Thermi, where the laboratory was housed. Thirty five seedlings of each variety were transplanted into another yellow star thistle garden (in a young olive grove) where they were interplanted with safflower (Carthamus tinctorius L.) in a 7 x 7 Latin square planting and 8 artichoke plants were randomly added later. This planting was designed as a host selection field test to see if Urophora siruna-seva (H.G.) (a trypetid fly natural enemy of yellow starthistle) would also select safflower as a host plant. The plants in this trial (245) were examined every 10 days from May 1 to October 1 and data on the stage of the plants, the condition and mortality of the plants as well as insects observed on the plants and insect damage was recorded.

In all about 10,500 bits of data were recorded, and about 100 insects (11 species) mostly curculionidae were collected pinned and prepared for identification. These have not yet been sent, nor have the data on plant growth and

mortality been analyzed.

# Urophora siruna-seva

The 7 x 7 Latin square with artichokes inter-planted also served as an experiment to test the Greek strain of Urophora on safflower and artichoke. The planting was located in an area where Greek YST was quite common. The plants were examined every 10 days, from May through August and  $\underline{U}$ .  $\underline{siruna-seva}$  was never observed on safflower, even though frequent observations were recorded on YST, both Greek and US strains.

On July 8 when the flower heads were mature, 105 heads were collected (3 from each plant in each square = 15/square) and of these 100 were dissected. Another 100 were collected on the same date from Greek YST plants growing

nearby. Table 1 shows the results of the dissections.

The 8 artichoke plants growing among the YST plants in the garden did not flower in 1982 so it was not possible to collect mature heads from them. However mature artichoke heads were collected in two locations that were also infested with yellow starthistle plants, the yellow starthistles being as close as 1 meter to the artichoke plants.

One artichoke location was only about 100 meters from the test plot. On July 22 10 large and 14 small artichoke heads were collected from this plot.

From the second plot at Triadi (4 km distant) two large and 1 small artichoke heads were collected and dissected. One hundred plus YST heads were collected within a radius of 4 meters of each of these artichoke sites. Dissection of the artichoke heads disclosed no <u>U. siruna-seva</u> galls or larvae while the YST heads from both plots were infested.

Artichoke, as was expected, when exposed to natural populations of YST is not a host plant. In Table 1 it is noted that two galls were found in safflower plants in the  $7 \times 7$  Latin square. This spurred the interest to see if and

how heavy the attack of gall making trypetids was on safflower, so another 200 heads were dissected and 16 galls were found. These galls were suspect as not

being U. siruna-seva because they were larger than normal.

Dr. Helmut Zwolfer (University of Bayreuth, Germany) was visiting Greece at the time and he was inclined to believe the galls were not caused by  $\underline{U}$ .  $\underline{siruna-seva}$  but by  $\underline{U}$ .  $\underline{macrura}$ , which has been reported from a plant in the same genus as safflower ( $\underline{Carthamus}$  lanatus L.). (In any event, the flies emerged in late January 1983 and were not  $\underline{U}$ .  $\underline{siruna-seva}$ , so the flies galling the YST heads can be sent to California).

During the period from August through October, several thousands YST heads from plants of US origin were dissected and 300 galls were collected for shipment to California. The galls were treated with Tedion to kill the  $\underline{\text{Pyemotes}}$  mites and were placed in a cold cabinet to be kept at  $8^{\circ}\text{C}$ , until they are removed in the spring of 1983 to be sent to California for emergence and release.

During dissection of YST heads to recover the <u>siruna-seva</u> galls, it seemed that some plants were more susceptible to infestation than others. For example a single CA/W plant at the institute garden had 10% of the heads infested, while other CA/W plants and plants from other US locations had 0-4% infestation.

In an attempt to quantify this observation, some of the individual YST plants from the US that were planted in the  $7 \times 7$  Latin square plot and also some Spanish plants were examined (40-100 flower heads/plant). The results of this

dissection appear in Table 1.

All plants were of the same age and grown in the randomized 7 x 7 Latin Square (except the spanish plants, which were grown in a YST garden, near the latin square plot). Considering the uniformity of age and the randomized planting arrangement, the variation could be due to genotypic variation of the plants. On the basis of these observations, seeds of CA/W (the variety most infested) were returned to California to be planted for the  $\underline{U}$ . Siruna-seva release, hopefully to insure establishment when the fly is liberated. Subsequently, comparisons of the flies acceptance of plants from other localities can be measured to determine if there is a difference in susceptibility between them.

As a result of dissecting thousands of YST heads it seemed that some plants were more susceptible to <u>Urophora</u> attack than others. For example 10% of the heads in one California  $(\overline{W})$  plant in the garden were infested while the other plants of the same biotype, as well as all other US YST plants, had 0-4% of their heads infested. Table 1 provides some indication of the relative suscep-

tibility of YST biotypes to Urophora attack.

Table 1. Results of dissecting flower heads of yellow starthistle plants to determine relative susceptibility of different biotypes to <u>Urophora</u>

attack					
	No. Plants	No. Plants	No Heads ^	No. Galls	Total
Plant	Examined	Infested	Dissected	per plant	No.galls
California W	14	4	905	1 to 7	14
California P	19	2	1630	1	2
Washington 1	18	6	1325	1 to 9	20
Washington 5	13	1	1140	4	4
Spanish YST +	6	4	550	1 to 20	36

<sup>\* 40</sup> to 100 flower heads were dissected per plant.

<sup>+</sup> Plants were growing outside but adjacent to plot area.

Seed from the California (W) plant supporting the highest infestation of  $\underline{U}$ . siruna-seva was mailed to cooperators in Albany, California with a suggestion that the Greek strain of this fly be first established in California on plants grown from this seed.

# Bangasternus orientalis Capiomont (Coleoptera: Curculionidae)

The research on this insect has been divided between Greece and Rome; the biology to be done in Greece and the host specificity testing in Rome. The bulk of the investigation accomplished thus for is included in the petition for introduction of Bangasternus into quarantine, and is part of this report.

Host Specificity Experiments not included in the Petition for introduction,

Greek Field Test: After eggs were first found on yellow starthistle in late-May, 8 artichoke (Cynara scolymus) and 35 safflower (Carthamus tinctorius L.) plants were examined every 10 days throughout the beetles oviposition period to see if eggs would be oviposited on them. These arthichoke and safflower plants were within a 5 m radius of the yellow starthistle plants that served as oviposition sites for females. The beetles did not lay any eggs on either artichoke or safflower plants.

In the laboratory attempts were made to force <u>Bangasternus</u> females to oviposit on safflower buds. This was done by decorating the buds with spines to simulate YST buds and coating them with an extract of YST buds. The females failed to

oviposit on them.

It was noticed that the female palpates the curved hairy surface of the young plant tips with the tip of her abdomen prior to laying an egg. The surface features or shape of this young growth may provide a cue to encourage oviposition.

Bangasternus orientalis is a very promising biocontrol agent for C. solstitialis

for the following reasons:

1) All field observations, larval feeding trials and oviposition tests indicate that B. orientalis is specific to yellow starthistle.

2) The oviposition period of Bangasternus lasts for about 2 months and is well synchronized with the flowering of  $\underline{C}$ . solstitialis.

3) A single larva can nearly destroy all the seeds in a YST flowerhead.
4) The distribution of B. orientalis coincides well with the distribution of YST from sea level to 850 m above sea level.

# Bruchidius tuberculatus Hochh. (Coleoptera: Bruchidae)

This bruchid is one of the most effective natural enemies of YST in Greece where it attacks the flower heads late in the season and destroys seeds in previously unattacked heads. It will also destroy any remaining seed in heads

previously attacked by trypetid flies or curculionid beetles.

A large segment of time was spent studying the biology and host specificity of this insect and it all looked very promising until September 11, when 5 adult Bruchidius were found in the heads of safflower plants in the 7 x 7 Latin Square. Also, when Bruchidius larvae were transferred to safflower seeds they completed their development. Based on these findings, B. tuberculatus has been dropped from the list of possible candidates for the biological control of YST.

#### Fungus diseases

Two fungal pathogens of <u>C. solstitialis</u>, tentatively identified as <u>Puccinia centaurea</u> (DC) Mart. and <u>Erisyphe cichoracearum</u> DC, were found on YST in the garden at the Plant Protection Institute. These pathognes also attacked the US yellow starthistle varieties planted at the Institute. Specimens of attacked plants were sent to Drs. Defago, ETH, Zurich and B. Bruckhart, USDA, Plant Disease Research Laboratory, Frederick, MD.

Summer Rainfall Mortality: This year there was substantial rain from August to October. Conditions were right for quick drying because these rains were in the form of short showers followed by wind and sun. This quick drying precluded the germination of YST seeds, so the high, summer rain induced seed mortality seen in 1981 was not present this summer. The first YST seedlings were found in the last half of October. By that time it was cooler so seedling survival was quite high in 1982.

Survey of New Candidates: Between mid-February and mid-May over 3,000 rosettes of YST were examined for candidate biocontrol agents. Other than Apion sp., the following organisms were encountered:

- a) <u>Sphaeroderma rubidium</u> Graells. (Coleoptera: Chrysomelidae). This is a known artichoke pest and fed on safflower in a feeding trial.
- b) Three species of leaf mining flies. Two of these were reared to adults and sent for identification. The third pupated in soil but no adult was produced. All three species were heavily parasitized.
- c) A leaf-hopper. It damages potted YST plants and was sent to Rome to be forwarded for identification.
- d) On April 3 larvae of a curculionid species were found feeding in fusiform galls near the root-necks of YST plants near Alexandropolis. About 100 of these galls were collected. Some of these larvae were transferred to yellow starthistle and safflower stems, but they fed poorly in both hosts and only two larvae were reared to adults; these were sent to Rome for identification. It is a <u>Lixus</u> species and is probably not a good candidate.

Later in the season, <u>Bangasternus provincialis</u> Fairmaire and <u>Larinus minutus</u> Gyllenhal were found on <u>yellow starthistle</u>, which is probably a <u>marginal host</u> of these insects. Both of these insects were found in large numbers on diffuse knapweed, <u>Centaurea diffusa</u> Lam.

An extensive search was made for <u>Eriophyes centaurea</u> (Acar: Eriophyidae) and <u>Cyphocleonus morbillosus</u> F. both reported in the literature as occurring on YST on mainland Greece. Neither was found.

An eriophyid mite probably <u>E. centaurea</u>, was collected from <u>Centaurea scabiosa</u> L. in Germany by Professor Helmut Zwolfer and sent to Greece. Several attempts were made to colonize these mites on YST under varying conditions with no success. It is not clear if the mites were stressed by the trip, if they are too specific, or if the experimental techniques were faulty.

# Centaurea diffusa Lam.

This plant is very common around Thessaloniki. The curculionids  $\underline{B}$ .  $\underline{provincialis}$  Fairmaire,  $\underline{Larinus}$   $\underline{curtus}$   $\underline{Hochh}$ ., and  $\underline{Larinus}$   $\underline{minutus}$   $\underline{Gyllenhal}$  are  $\underline{common}$  on  $\underline{C}$ .

In a small experiment some  $\underline{B}$ . provincialis adults were caged with  $\underline{C}$ . solstitialis. They fed and layed eggs with no apparent problem.



#### APPENDIX II

A Petition for the Introduction into Quarantine for Further Testing the Weevil Bangasternus orientalis Capiomont (Coleoptera: Curculionidae), a Candidate Natural Enemy for the Biological Control of Yellow Starthistle (Centaurea solstitialis).

Prepared by:

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and

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and

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#### Introduction

Yellow starthistle, <u>Centaurea solstitialis</u> L., is scattered throughout much of the United States and in Canada from Ontario to British Columbia (Maddox 1981). Maddox (1981 and references therein) discussed the great economic importance of this weed in California where it has invaded certain cultivated crops, pastures and rangelands, and roadsides and wastelands. The weed has also become a serious threat to pasture and rangeland productivity in Oregon, Washington, and Idaho.

Biological control offers potential as a self-perpetuating and non-disruptive method of control for yellow starthistle. Thus, the USDA Biological Control of Weeds Laboratory-Europe is studying the bionomics and host plant specificity of several natural enemies of this weed in its native range with the aim of clearing and introducing into North America the most promising of these natural enemies. Earlier efforts by University of California entomologists led to the introduction in 1969 of the seed fly Urophora siruna-seva (Hering) into California, but it failed to become established on California plants. Efforts to match a Greek strain of this fly with North American biotypes of yellow starthistle have been successful, just as they have been in finding other promising biological control agents of which the seed weevil Bangasternus orientalis Capiomont (Coleoptera: Curculionidae) is one.

### Taxonomy, Geographic Distribution and Host Plants

Family: Curculionidae
Subfamily: Cleoninae

Tribe: Lixini

Genus: <u>Bangasternus</u> Des Gozia, 1886

Species: <u>orientalis</u> Capiomont

Length of beetle: 5-6 mm

Color: Brownish hue with a tint of black and white

Identification was first made by Enzo Colonnelli, (Istituto di Zoologia dell'Universita' di Rome, Italy) and later by Dr. D.R. Whitehead, Systematic Entomology Laboratory, IIBIII, USDA.

There are 9 recognized species in the genus (based on a survey of the taxonomic literature by Enzo Colonnelli, curculionid specialist, for the USDA Rome Lab.). These species and their recorded Palearctic distributions and host plants are

given in Table 1.

Centaurea iberica and safflower were listed by Ter-Minasyan (1978) as host plants of  $\underline{B}$ . Orientalis. It is important to mention, however, that this weevil did not accept safflower as a host in the 1982 specificity tests conducted at the Rome Laboratory and in Greece. The other plant ( $\underline{C}$ . iberica) was not tested; however, the weevil did not accept Centaurea cyanus in Rome tests. This is shown below in the section on host specificity experiments.

We emphasize that the genus Bangasternus has received little taxonomic and

biological attention.

# Life History

Studies of the life history of this weevil are still in progress at Greece and Rome. From the field studies conducted in 1982 in Thermi, Greece it is possible to provide a general description of the life history of  $\underline{B}$ . Orientalis for this petition.

This species overwinter as adults. The first adults of the 1982 season were found on May 10 resting alone on yellow starthistle plants. The first pairings

were observed on May 17 on new growth of yellow starthistle. Throughout the rest of May and June it was possible to find and collect the fairly abundant adults. The last adults were seen in the field on July 29. Feral adults were found feeding only on the stem wings of yellow starthistle. Adults will nibble on leaves, albeit minimally, when they are confined with leaves in the laboratory.

In the field the oviposition period lasts about 2 months and is well synchronized with the bud stage of its host plant. When mid-day temperatures are high

in June and July the adults rest in shade on lower parts of plants.

The following account of the oviposition behavior of B. orientalis is based on careful observations in the field in Greece. For oviposition, females select branch tips supporting young floral buds (Bu-1 and Bu-2 of Maddox 1981; also, some branch tips without a well-defined bud may be selected but bud development then occurs within 2-days). The female walks up and down several branches searching for a suitable bud. When she finds a suitable bud she walks to its tip, turns around and moves down the stem a few centimeters, then walks backward to the tip of the branch and examines the bud and surrounding leaflets, searching for a suitable location to lay an egg. When the right bud is discovered, she probes the leaflet covering the bud with the tip of her abdomen. When the correct site is found she stops for a few minutes, keeping the ventral tip of her abdomen in contact with the plant. When she is ready she deposits one egg at a time along with dark green mucous material that forms a protective egg cap. After this action, she moves down the stalk a few millimeters, drawing the mucous material to a point and breaking it. The cap is dark green and soft at first, but with exposure to the air it gets darker and harder until it becomes black and very hard.

When a female is finished laying her egg, she moves downward a few centimeters, rests, and normally feeds a little; sometimes, however, a female will immediately start searching for another suitable oviposition site. In one instance a female layed an egg then she moved to an adjacent branch, walked to the apex and flew This behavior would seem to be favorable for the distribution of the away.

insect.

On each occasion, only a single egg was layed per oviposition site by a female. We are led to the conclusion that other females will lay eggs on a bud previously selected by a conspecific because up to 8 eggs have been found on one bud.

The eggs are usually placed on the leaflets surrounding the bud, sometimes on the plant stalk near the bud, and seldom directly on the bud. In those instances when a branch tip without a well-defined young bud is selected, the egg is placed on a leaflet surrounding the site which will support a new bud within 2-days.

The oviposition instinct or drive is apparently very strong. Often females searching for oviposition sites are disturbed by males, but they go right ahead

with their searching or egg laying.

Neonate larvae mine down the leaves and enter the bud, completing their life cycle there, consuming the seeds. Without a well defined capitulum or composite-

like seed head the larvae cannot complete their development.

We do not know the length of the larval development period under field conditions or under controlled laboratory conditions but this aspect will be studied. The larvae pupate in the flower heads. The length of the pupal stage is unknown. However, there is one generation per year in Thermi, Greece.

# Mortality Factors

Two species of egg parasitoids have been recovered from Greece. These were identified by Dr. D.L. Vincent, Beneficial Insect Introduction Laboratory, USDA, as a species of Mymaridae (species not known) and Pterandrophysalis levantina

Nowick: (Hymenoptera: Trichogrammatidae). Also, a <u>Pyemotes</u> mite has been found under the elytra of some adults. Its effect on the weevil is unknown.

At this time nothing more can be said about other biological factors which could influence the survival of this species.

### Effect of Organism on Host Plant

The larvae, as stated above, are seed feeders. In the field in Greece a single larva will almost completely destroy the seeds in a flowerhead. Quantitative data is not available but this aspect is under study.

### Potential Control Value

Since this is a petition for the introduction of  $\underline{B}$ . Orientalis into the Albany, California quarantine for further testing, it would be premature to attempt to rate its potential value as a biological control agent.

### Host Specificity Experiments

Greek Field Test: After eggs were first found on yellow starthistle in late-May, a number of artichoke (Cynara scolymus) and safflower (Carthamus tinctorius) plants were examined on a regular basis throughout the beetle's oviposition period in order to see if eggs would be oviposited on these two plant species in the field. The 8 artichoke and 35 safflower plants were within a 5 m radius of the yellow starthistle plants that served as oviposition sites for females. Visable examination of these plants at least every 10 days revealed that the beetles did not select artichoke and safflower plants as oviposition sites, as no eggs were found on these plants.

Rome Single Plant (No-Choice) Oviposition Test: Two-hundred adults of  $\underline{B}$ . Orientalis were collected in Greece by Dr. Sobhian and sent in May to the Rome Laboratory. This material was used to conduct this test and a first instar larval survival test (see below).

The plants selected for this test are listed in Table 2. There were 5 replicates per test plant. The potted plants were placed in the laboratory garden where they were each covered with a transparent plastic cylinder (dia. 20 cm; height 70 cm). There were four organdy covered holes (dia. 10 cm) in the sides of each cylinder to permit some air circulation. Each cylinder was capped with organdy cloth held in place with a large rubberband. On 17 June each caged plant received 2 females and 2 males of  $\underline{B}$ . Orientalis. On 5 July, when most of the adults were dead, all plants were examined under a stereo microscope for evidence of oviposition.

During the experiment the ambient temperature was  $15-35^{\circ}$ C, RH was 40-90%, and photoperiod was ca. 15 h. Temperature and humidity inside the caged plants was not measured.

The results in Table 2 are very encouraging. All of the <u>C</u>. solstitialis biotypes were suitable hosts. Eggs were not found on any of the other plants included in the test.

Rome First Instar Larval Survival Test: The same plant species used in the preceding test were used in this test. One bud on each of 10 plants (= replicates) was infested with 4 fertile eggs (total of 40 eggs per test plant). A fine camel's hair brush was used to transfer the eggs, which were ready for eclosion (mandibles visible), to an area between the bracts of the immature buds of the

test plants. Each infested bud was marked so it would be possible to record egg hatch.

Buds were infested between June 10-15. All of the eggs hatched. The flower heads were dissected under a stereo microscope on 13 and 14 July, at which time

the number of larvae was recorded.

The results of this test, as shown in Table 3 provide additional evidence for the strict host plant specificity of  $\underline{B}$ . Orientalis for yellow starthistle. Only one larva can survive in a flower head, an aspect we discovered as a result of this test and field studies in Greece.

Proposed Host Specificity Tests for 1983 in USDA Quarantine, Albany, California: Although major testing will be done in 1983 at the Rome Laboratory, important tests should also be conducted simultaneously in Albany. The Albany work will focus on native U.S. plants closely related to yellow starthistle. This testing will be conducted by Mr. Don Maddox, Research Entomologist, USDA Biological Control of Weeds Laboratory, Albany, California. The proposed Albany test plant list is as follows:

Centaurea americana
Cirsium sp. 1
Cirsium sp. 2

Native U.S. species Native-endangered U.S. species Native-endangered U.S. species

The availability of seed will govern the selection of the various species, so it is not possible to give the exact species at this time. A representative number of native-endangered species will be tested, however.

# Discussion and Summary

At present, we feel there is little risk associated with bringing  $\underline{B}$ . orientalis into quarantine at Albany to ascertain the host plant specificity of this weevil for selected U.S. plants closely related to the target weed. If the insect is brought into the Albany quarantine, tests can be conducted simultaneously to those in Rome and perhaps this will reduce the pre-release testing time, if all the results are favorable.

In sum, on the basis of the tests outlined in this petition we are going ahead with more in-depth studies of  $\underline{B}$ . orientalis. It is important to point out that the beetle only accepted the various biotypes of  $\underline{C}$ . solstitialis in all of the tests run to date.

Thank you for the consideration.

### Bibliography

- Maddox, D.M. 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and central valley situations in California. Agricultural Research Service, USDA, Agricultural Research Results (Western Series), No. 20, 33 pp.
- Ter-Minasyan, M.E. 1967. Weevils of the subfamily Cleoninae in the fauna of the USSR, tribe Lixini. Academy of Sciences of the USSR; Keys to the USSR Fauna published by the Zoological Institute, Academy of Sciences, USSR. Nauka Publishers, Leningrad. (translated into English for ARS).

Table 1. Tabular summary of biological information on Bangasternus.

Species of <u>Bangasternus</u>	Known Geographic distribution	Recorded Host Plants
araxis Reitter	Caucasus Mountains and Turkestan (USSR); Central Asia	unknown
<u>diecki</u> Capiomont	Southern Spain	unknown
<u>fausti</u> Reitter	Araxestal (Caucasus) USSR; Armenia	Carthamus sp. Centaurea squarros
orientalis Capiomont smyrnensis Cap.	Southeast Europe including Austria; Balkans; Asia Minor including Turkey; Caucasus Mountains and Turkestan (USSR); Israel	Centaurea / 2/ soltitialis / 2/ Centaurea iberica
planifrons Brulle'	Eastern Mediterranean; Turkmenia; Greece; Asia Minor; Syria	unknown
provincialis Fairmaire	France, Italy	Centaurea nigra, C. paniculata; C. scabiosa
siculus Capiomont	Sicily; Spain	unknown
syriacus Stierlin	Syria	unknown
villosus Capiomont hispanicus Cap.	Spain, Morocco	unknown

<sup>1/</sup> This record is a result of the research discussed in this petition.

 $<sup>\</sup>frac{2}{\text{discussed in this petition.}}$  is another new host record. This is the result of research

Table 2. Results of a single plant (no-choice) oviposition test with adults of <u>Bangasternus orientalis</u>, Rome, Italy, 1982.

Replicate No.	1	2	3	4	5	Total eggs
Plants tested		No. egg	s at di	ssectio	n	collected
Centaurea solstitialis (control) Greece	107	40	39	139	79	404
C. solstitialis (WA-USA) Golden dale	69	37	35	62	105	308
C. solstitialis (WA-USA) Walla-Walla	56	70	57	59	75	317
C. solstitialis (WA-USA) Yakima	30	90	42	38	57	257
C. solstitialis (CA-USA) Concord	61	35	83	20	40	239
C. solstitialis (Italy-Bracciano)	41	51	39	60	48	239
C. cyanus (Italy)	0	0	0	0	0	0
Helianthus annuus (USA)	0	0	0	0	0 ,	0
H. tuberosus (Italy)	0	0	0	0	0	0
<u>Calendula officinalis</u> (Italy)	0	0	0	0	0	0 a
Zinnia <u>elegans</u> (Italy)	0	0	0	0	0	0
Chrysanthemum leucanthemum (Italy)	0	0	0	0	0	0
Carthamus tinctorius (USA)	0	0	0	0	0	0
Cynara scolymus (USA)	0	0	0	0	0	0
Cynara scolymus (Italy)	0	0	0	0	0	0

<sup>1/</sup> Experimental details are given in the text.

Table 3. Results of a preliminary survival test of first instar larvae of <u>Bangasternus</u> orientalis on various test plants.

Replicate No.	1			4	5		7	8	9	10_	Total larvae	% larvae survived
Plants tested		1	10. ]	larva	e a	live	at c	lisse	ctio	n	collected	
Centaurea solstitialis (control) Greece	12/	1	1	1	1	1	1	1	1	1	10	100
C. solstitialis (WA-USA) Golden dale	1	1	1	1	1	1	1	1	1	1	10	100
C. solstitialis (WA-USA) Walla Walla	1	1	1	7	1	1	1	1	1	7	10	100
C. solstitialis (WA-USA) Yakima	1	1	1	1	1	1	1	1	1	1	10	100
C. solstitialis (CA-USA) Concord	1	1	1	1	1	1	1	1	1	7	10	100
C. solstitialis (Italy)	1	1	1	1	0	1	1	1	7	1	9	90
C. cyanus (Italy)	0	0	0	0	0	0	0	0	0	0	0	
Helianthus annuus (USA)	0	0	0	0	0	0	0	0	0	0	.0	
H. tuberosus (USA)	0	0	0	0	0	0	0	0	0	0	0	-
Calendula officinalis (Italy)	0	0	0	0	0	0	0	0	0	0	0	
Zinnia elegans (Italy)	0 、	0	0	0	0	0	0	0	0	0	0	
Chrysanthemum leucanthemum (Italy)	0 0	0	0.	0	0	0	0	0	0	0	0	
Carthamus tinctorius (USA)	0	0	0	0	. 0	0	0	0	0	0	0	
Cynara scolymus (USA)	0	0	0	0	0	0	0	0	0	0	0	
Cynara scolymus (Italy)	0	0	0	0	0	0	.0	0	0	0	0	

<sup>1/</sup> Experimental details are given in the text.

<sup>2/</sup> Based on field and laboratory observations only one larva of <u>Bangasternus</u> orientalis can survive in a flower head of <u>Centaurea</u> <u>solstitialis</u>.

APPENDIX III

Petition for the Introduction of <u>Pterolonche inspersa</u> Stgr. (Lepidoptera: Pterolonchidae) into Quarantine at Albany, California.

Prepared by:

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and

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#### Introduction

According to Maddox  $(1977)^{\frac{1}{2}}$  diffuse knapweed infests an estimated area in excess of 3,500,000 acres in the states of Washington, Oregon, Montana and Idaho. An infestation of this magnitude is clearly out of control and cannot be arrested by chemical means without the expenditure of millions of dollars, if even then.

As a result of the large infestations on land of low yield a biological control program has been started in Canada and the United States and Pterolonche inspersa is one of the complex of insects stated for introduction as a candi-

date biological control agent against diffuse knapweed.

### Taxonomy

The genus <u>Pterolonche</u> is a small genus of a few species that comprise the family Pterolonchidae according to Gozmany, (Museum Allattara, Budapest) which is separate, but closely related to the family Gelechiidae.

The insects from the Greek population with which we are working have been positively identified as <u>Pterolonche</u> inspersa by Dr. H.J. Hanneman at the

Zoologishes Museum Humboldt, University of Berlin.

While there is some taxonomic confusion at the family level, there seems to be no problem of determination at the species level.

# Geographic Distribution

The recorded distribution for this insect is Spain, France, Soviet Union, Hungary, Greece, Turkey (Anatolia), Bulgaria, Romania and Italy. The Italian records are very old, because there are no known Centaurea diffusa or  $\underline{C}$ . maculosa populations currently known in Italy.

# Host Plants

The insect is known only from <u>Centaurea</u> spp. According to unpublished information of Prof. Helmut Zwolfer, <u>University</u> of Bayreuth, W. Germany, <u>Pterolonche inspersa</u> was collected from <u>Centaurea diffusa</u>, <u>C. maculosa and C. paniculata in surveys made in Northern Greece between 1961 and 1971.</u>

Examination of the Review of Applied Entomology (1918-1981), and the Zoological Record (1920-1970) provided no host plant records for this insect, indicating that it is not a known agricultural pest. In fact, prior to the study of the biology of the insect by Campobasso the host plant was unknown to the

taxonomic specialists of this group of insects.

Campobasso was collecting <u>Pterolonche</u> in northern Greece. About 50 plants each of <u>Onopordum spp.</u>, <u>Cirsium spp.</u>, <u>Sonchus and Carduus spp.</u> growing in the area were dissected and examined for the presence of <u>P. inspersa larvae</u>. No larvae were found in any of these nearby related plants, thus the preliminary information suggests that the insects are specific (stenophagous) to the genus Centaurea.

 $<sup>\</sup>frac{1}{2}$  Proc. Knapweed Symposium. Kamloops, BC Oct. 6-7, 1977. pp. 271-275.

# Biology and Life History

The information on the life history and biology presented here is based partly on laboratory findings. The field information was gotten during a survey and two collecting trips - to Greece in June and July 1979, 1980, 1981 and the laboratory information comes from observations made in Rome during the rest of the year in 1980 and 1981.

#### Oviposition

The eggs of  $\underline{P}$ . inspersa have been seen only in caged trials, and in these trials they were laid indiscriminately in the cage on the walls on plants, etc. In nature it is supposed that they are more or less randomly placed on the Centaurea diffusa plant during the first decade of August.

#### Larvae

The first instar larvae, after eclosion, feed down the root of the plant, some mining the woody portion and others feeding in the epidermis. The feeding location of the larva probably depends on where the egg was laid on the plant, ie. larvae from eggs put near the center of the plant probably mine the woody portion of the root while those eggs that were placed on the peripheral portion of the plant most likely produce larvae that feed on the outside layer of the root.

Based on the observations made in Rome, the larvae probably feed until about half grown (3rd instar) and overwinter in that stage. When the weather warms in the spring the larvae start to feed again, probably pupating in early July and emerging during the last half of July to mate, lay eggs, etc. making it a univoltine species. The roots of the knapweeds are small by nature, so many are seriously damaged by the one or two larvae feeding in each plant that make galleries 3-5 cm long and 2-2.5 cm wide.

In addition, some observations were made on the length of the pupal, adult and egg stages of the insect under outside conditions. These findings are

presented in Table 1.

Very likely when the larvae start to feed, they spin a characteristic silken tube lining the gallery they have made or covering the area they have fed on. The larvae stay in the tube, increasing its size as they grow. Eventually the tube is spun with an opening near soil surface, and the larva overwinters in the tube and pupates in the tube just below the soil surface, thus the tube offers both protection during the pupal stage and easy exit route from the soil for the emerging adult.

# Host Specificity Tests

Two different tests were made to determine the host specificity of <u>Pterolonche</u> and acceptance of American biotypes of diffuse knapweed. The first of these was an oviposition choice test and the second was a first instar larval survival test.

### Oviposition and Choice Test

In order to determine the oviposition preference, if any, 799 and 766 adult P. inspersa were confined for 10 days in each of four cages with the following potted plants: Centaurea diffusa (Greece) control; Centaurea diffusa (USA);

Centaurea solstitialis; Centaurea cyanus; Zinnia elegans.

The test cages were  $90 \times 90 \times 90$  cm and  $90 \times 90 \times 160$  cm and two of each size were used (2 replicates). The cages were kept in the garden and were large enough to permit the adults to fly inside and select plants for oviposition. The results of this trial are presented in Table II and III.

### First Instar Larval Survival Test

To find the acceptability of the following plants as hosts, 5 potted plants (replicates) of each of the following species were each infested with 5 newly hatched first instar larvae (25 larvae/each test plant species).

Centaurea diffusa european (control); Centaurea diffusa (USA) control; Cynara scolymus; Carthamus tinctorius; Helianthus annuus; Helianthus tuberosus; Aster chinensis; Cichorium intybus; Achillea millefolium; Tanacetum vulgare; Centaurea

solstitialis and Centaurea cyanus.

During the experiment all the plants were kept out of doors, under natural conditions and the test lasted from August 17 until September 30, when the experiment was stopped. All plants were then dissected under a stereo microscope and the surviving larvae were counted, collected and stored in ethyl alcohol. The results are presented in Table IV.

### Discussion

Larvae survived only on the  $\underline{C}$ .  $\underline{diffusa}$  control. Two larvae were recorded from each of the 5 replicates in the European  $\underline{C}$ .  $\underline{diffusa}$  control and two larvae were recovered from all but one of the 5 replicates in the U.S.  $\underline{C}$ .  $\underline{diffusa}$  control for a total of 19 larvae from the two controls.

Thus, 33% of the larvae used to infest <u>C</u>. <u>diffusa</u> controls were recovered compared to no larval recovery from any of the other plants in the test.

#### Recommendation

The fact that first instar larvae of  $\underline{P}$ . inspersa completed development on the diffuse knapweed controls and failed to develop on any of the test plants including the cultivated thistle artichoke and safflower and the composite sunflower indicate that it is safe to bring the insect into quarantine for further testing without posing any significant risk to American agriculture.

Therefore, concurrence is requested from the Joint Working Group for Biological Control of Weeds, the State of California and the University of California

to import this candidate into quarantine at Albany, California.

Table I. Biological data of Pterolonche inspersa Stgr. July-September 1981.

•		Laboratory reared adults			
		. ( <del>x</del>	+	SD)	
Pupal stage (20 insects) $\mbox{\ensuremath{\upalpha}}$ longevity (10 $\mbox{\ensuremath{\upalpha}}$ ) $\mbox{\ensuremath{\upalpha}}$ longevity (10 $\mbox{\ensuremath{\upalpha}}$ ) $\mbox{\ensuremath{\upalpha}}$ reoviposition period $\mbox{\ensuremath{\upalpha}}$ oviposition period $\mbox{\ensuremath{\upalpha}}$ eggs hatched $\mbox{\ensuremath{\upalpha}}$ / eggs hatched $\mbox{\ensuremath{\upalpha}}$ /	(days) (days) (days) (days) (days) (days)	14.7 15.8 10.7 2.6 7.4 142.2	36%	2.4 2.6 1.4 0.8 2.2 59.2 4.7	

<sup>1/</sup> Eggs in biological study reached blackhead stage, but failed to hatch. Data for % hatch were taken from 1249 eggs collected in field cages.

Table II. Oviposition choice test, small cage (90  $\times$  90  $\times$  90 cm).

Test plant	Rep I	No. eggs Rep II	Total
Centaurea diffusa (Greece - control) Centaurea diffusa (USA) Centaurea cyanus Zinnia elegans	208 171 12 1 0	195 199 0 0	403 370 12 1 0
Total	392	394	746

Note: From a total of 766 eggs collected, 342 hatched = 44%.

Table III. Oviposition choice test, large cage (90  $\times$  90  $\times$  160 cm).

Test plants	Rep I	No. eggs Rep. II	Total
Centaurea diffusa (Greece - control) Centaurea diffusa (USA) Centaurea solstitialis Centaurea cyanus Zinnia elegans	.134 129 0 0	46 109 0 0	180 238 0 0
Total	263	155	418

Note: From a total of 483 eggs collected 111 or 22% hatched.

Table IV. First instar larval survival trial (Plants dissected 30 days after infestation).

Replicate No. Plants tested	1 No.1	2 arvae	3 alive	4 at dis	5 section 1/	Total larvae alive
Centaurea diffusa (Greece)	2	2	2	2	2	. 10
C. diffusa (USA)	2	2	2	2	1	9
Cynara scolymus	0	0	0	0	0	0
Carthamus tinctorius	0	0	0	0	0	0
Helianthus annuus	0	0	0	0	0	0
Helianthus tuberosus	0	0	0	0	0	0
Aster chinensis	0	0	0	0	0	0
Chicorium intybus	0	0	0	0	0	0
Achillea millefolium	0	0	0	0	0	0
Tanacetum vulgare	0	0	0	0	0	0
Centaurea solstitialis	0	0	0	0	0	0
Centaurea cyanus	0	0	0	0	0	0

<sup>1/</sup> Five 1st instar larvae placed on each plant replicate.

# APPENDIX IV

Host Specificity Tests of Pterolonche inspersa (Lep.: Pterolonchidae) a Candidate for the Biological Control of Diffuse Knapweed Centaurea diffusa. //

Investigator: Gaetano Campobasso

In 1981 a sufficient number of just hatched first instar larvae of this moth were obtained to do a preliminary host specificity test. Twelve plant species, including the controls, ( $\underline{\text{Centaurea}}$  diffusa, Greece and  $\underline{\text{C}}$ . diffusa USA) were tested.

### Test Procedure

Five healthy potted plants of each of the 12 species were used (total 60 plants). Each plant was considered a replicate, therefore each plant tested

was replicated 5 times.

Five just hatched first instar larvae were transferred to each plant with a sable brush. The larvae were placed near the central whorl of leaves of the  $\underline{\text{C}}$ .  $\underline{\text{diffusa}}$  rosette. Twenty five larvae were used for each plant species for a total of 575 larvae in the whole test.

The replication and larval placement procedures for the 1981 and 1982 tests

were the same.

In the 1981 tests, the plants were dissected one month after infestation. Dissection at 30 days was not ideal, because the larvae were small and therefore

difficult to see and required more work to complete the dissection.

In the 1982 trials, the larvae were allowed to remain in the plants at least 60 days prior to dissection. By this time the larvae were large enough (ca. 7.7 mm in length - mean of 10 larvae) and the damage made was apparent enough to find them easily, if they were present in the plant.

### Discussion and Results

1981 Test

The results of this trial were most encouraging because  $\underline{P}$ .  $\underline{inspersa}$  clearly fed on the US biotype of  $\underline{C}$ .  $\underline{diffusa}$  and it was clearly a very specific insect, warranting further study.

1982 Test

The results of the 1982 trials are most encouraging and show the insect to be extremely specific. Other than <u>C</u>. <u>diffusa</u> there was larval survival on only 3 other species, and they were all <u>Centaurea</u>. The low percentage of larval survival, compared to the control, indicates that something is not right. However, at the time of dissection those larvae recovered from <u>C</u>. <u>friderici</u>, <u>C</u>. <u>cineraria</u>, and <u>C</u>. <u>corsiana</u> appeared to be healthy, feeding well and the same instar of those developing on <u>C</u>. <u>diffusa</u>. The larvae have not yet been measured, but they are in alcohol and head capsule measurement will be made later.

There were 10 plants in this list of 50 that were completely out of synchrony with the insect. In July and August, when the insect is ovipositing, these plants are completely senesced in the field, and are therefore no longer acceptable as host plants. The only question raised about these plants concerned

<u>Cichorium endivia</u>. Since this can be a cultivated plant, and occur all through the growing season, it could conceivably be in an acceptable stage during the oviposition season.

<u>Cichorium intybus</u> matures late, and was included in the 1981 tests. The results are negative. There is no reason to suspect that <u>C</u>. endivia would be accepted

as a host, but it can be tested by growing plants out of season.

Plants accepted by sibling species of <u>Pterolonche</u> were not tested, because of the paucity of information about this insect genus. Even the host plant of <u>P. inspersa</u> was unknown (it was thought to be grass) prior to Mr. Campobasso finding it in C. diffusa.

Pterolonche inspersa (Lepidoptera, Pterolonchidae).

First instar larval survival trial (Plants dissected 30 days after infestation). 1981.

Replicate No. Plants tested	No.	2 larvae	3 alive a	4 t disse	5 ction <u>l</u> /	Larvae survived Total	%
Centaurea diffusa (Greece)	2	2	2	2	2	10	40
C. diffusa (USA)	2	2	2	2	]	9	36
Cynara scolymus (Italian)	0	0	0	0	0	0	
Carthamus tinctorius	0	0	0	0	0	0	
Helianthus annuus	0	0	0	0	0	0	
Helianthus tuberosus	0	0	0	0	0	0	
Aster chinensis	0	0	0	0	0	0	
Chicorium intybus	0	0	0	0	0	0	
Achillea millefolium	0	0	0	0	0	0	
Tanacetum vulgare	0	0	0	Ö	0	0	
Centaurea solstitialis	0	0	0	0	0	0	
Centaurea cyanus	0	0	0	0	0	0	

<sup>1/</sup> Five 1st instar larvae placed on each plant replicate.

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First instar larval survival test of <u>Pterolonche inspersa</u> (Lep.: Pterolonchidae) (Five 1st instar larvae placed on each plant replicate). 1982.

Replicate No. Plants tested	No.	2 Tarvae	alive a	4 it disse	ction	Total	% la surv
Centaurea diffusa (Greece)	2	4	3	3	4	16	64
C. diffusa (USA)	2	5	3	2	3	15	60
C. axillaris	٥	0	ñ	Ō	Û	n	
	0	0	0	0	0	0	
C. calcitrapa	1.	0	0	0	0 -	0	
C. cineraria C. corsiana C. cristata C. crithimifolia C. friderici C. jacea	1	0	0	0	0	1	
C. corsiana	l	U	U	U	U	1	
C. cristata	0	0	0	0	Ņ	0	
C. crithimifolia	0	0	0	0	0	0	
C. friderici	1	0	1	. 0	0	2	
C. jacea	0	0	0	0	0	0	
C. rhenana	0	0	0	0	. 0	0	
C. scabiosa	0	0	0	0	0	0	
C. splendens	0	0	0	0	0	0	
Cirsium discolor (USA)	0	0	Ô	Ô	0	Ô	
C. andrewsii (USA)	ñ	n	Õ	Õ	Õ	Ô	
C. lanceolatum	0	0	Ô	Û	Õ	Õ.	
C. occidentale (USA)	0	0	0	0	0	0	
C. palustre	0	0	0	0	0	0	
	. 0	0	0	0	0	0	
Carduus nutans	0 .	0	0	0	0	U	
C. acanthoides	0 .	U	U	U	0	0	
C. pycnocephalus	-	-	7	•	₹'	-	
Senecio paludosis	0	0	0	0	0	0	
S. cineraria	0	0	0	0	0	0	
S. jacobaea	0	0	0	0	0	0 -	
S. nemorense	0	0	0	0	0	0	
Xeranthemum annuus	0	0	0	0	0	. 0	
X. clyndroceum	-	-	-		_		
SiTene vulgaris	0	0	0	0	0	0	
S. armeria		_	_	Ĭ.	_	-	
S. nutans	0	Ω	0	0	0	n	
Cynara scolymus (USA)	0	0	0	0	0	0	
	0	0	0	0	0	0	
Lactuca virosa	U	U	U	,	U	. 0	
L. muralis	-	~	-	-	-	-	
L. perennis Cichorium endivia	U	U	0	0	U	0	
Cichorium endivia	-	-	•	-	•	-	
/iola ciocca ,	•	-	-	•	•	-	
Arthemis arvensis	-	-	<b>-</b> ,	-		-	
A. tinctoria	0	0	Ó	0	0	0	
Arthemisia altissima							
Dopordum acanthium	0	0	0	0	0	0	
Canavacum officinal	0	0	0	0	0	0	
araxacum officinale	U	U	U	U	U	U	
lagetes erecta	-	-	-	-	-	-	
eontodon crispus	0	0	0	0	0	0	
L. hispidus	0	0	0	0	0	0	
Chrysanthemum corimbosum	0	0	0	0	0	0	
Papaver somniferum	-	-	-	-	-	• 1	
uphorbia lathyris	0	0	0	0	Û	0	
Medicago sativa	0	0	0,	0	0	0	
Achillea lingustica	0	0	0	0	0	0	

<sup>\*</sup> Plants not synchronized with the insect (senesced and dry at oviposition period).

<sup>\*\*</sup> Endangered or native US plants.

Convolvulus arvensis //
R. Sobhian, Thessaloniki, Greece

Convolvulus arvensis L.: A sample of eriophyid mites (possibly Aceria sp. or Eriophyes sp.) were collected on May 2 near Larissa, Greece and sent to Albany, as requested by Dr. Rosenthal. A second trip was made to Larissa on June 23 but only a few mites were found. These were used for a preliminary test in Greece but failed to establish on C. arvensis (L.) Scop. growing on the

Institute's grounds.

On September 24 and 25 a third trip was made to Larissa, but no mites were found, only dried plants. After the rains started on October 15 a strong colony of an eriophyid mite was found on the University grounds west of Thermi near the airport. Infested plant material from this site was pinned on 7 bindweed plants in the Institute garden; 6 were colonized after 3 weeks. The mite infestation was very low on these plants which also supported a fungus. One plant attacked by the fungus had some galls containing hundreds of mites. Samples of these mites were sent to Rome for identification.

Clement, Mimmocchi, Cristofaro

The aim of our research was to determine the worth of two arthropods, <u>Tyta luctuosa</u> (Denis & Schiffermüller) (Lepidoptera: Noctuidae) and <u>Aceria sp.</u> (Acarina: Eriophyidae), as biocontrol agents for field bindweed, <u>Convolvulus arvensis</u> L. Based on the earlier findings of Dr. Sara Rosenthal, <u>USDA Albany Laboratory</u>, it was felt that these two organisms had potential as biocontrol agents for <u>C</u>. <u>arvensis</u>.

Tyta luctuosa (Denis & Schiffermüller), Methods -

Host Specificity Tests: A review of the literature showed that this noctuid is a common defoliator of <u>C</u>. arvensis in Europe; however, at least four other plants have been recorded as larval hosts (see Rosenthal 1978 and references herein). Rosenthal, however, found that larvae barely tasted other recorded

host plants in laboratory tests.

Emphasis was placed on determining larval survival and development on plant species closely related to <u>C</u>. <u>arvensis</u>. The plants tested were in the Convolvulaceae: four biotypes of <u>C</u>. <u>arvensis</u> (henceforth referred to as Rome biotype; Texas biotype; Stockton, <u>California biotype</u>; and Yreka, <u>California biotype</u>); seven sweet potato, <u>Ipomoea batatas</u> Monnet Lamarck, varieties (Painter, Jewel, Baker, Jersey, Centennial, <u>UC779</u>, Garnet); and eleven <u>Calystegia</u> R. Br. species native to North America (<u>C</u>. <u>macrostegia</u> (Greene) Brummitt, <u>C</u>. <u>purpurata</u> (Greene) Brummitt, <u>C</u>. <u>fulcrata</u> (Gray) Brummitt, <u>C</u>. <u>subacaulis</u> H.& A., <u>C</u>. <u>occidentalis</u> (Gray) Brummitt, <u>C</u>. <u>polymorpha</u> (Green) Munz, <u>C</u>. <u>stebbinsii</u> Brummitt, <u>C</u>. <u>longipes</u> (Wats.) Brummitt, <u>C</u>. <u>atriplicifolia</u> Hallier f., <u>C</u>. <u>collina</u> (Greene) Brummitt, and <u>C</u>. <u>malacophylla</u> (Jepson) Munz).

Rosenthal (1978) previously studied the ability of newly hatched larvae of T. luctuosa to feed and develop on some plants in the Convolvulaceae and in

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closely related families, and on plants recorded in the literature as hosts. However, her tests did not embrace  $\underline{C}$ . arvensis biotypes, a large number of sweet potato varieties, or a large number of closely related U.S. species.

All plants used in our tests were grown from seeds or tubers provided by Dr. Rosenthal. Seeds and tubers were initially sown in pots in March and April

1982.

Adults were collected throughout the 1982 flight season (May through September) at Ponte Milvio, Rome, Italy. This site is a small (about 0.5 hectares), periodically mowed, open meadow adjacent to a busy bridge-road intersection area near the Tiber river. Besides <u>C. arvensis</u>, the dominant plants were clover and mallow. Adults were brought to the Rome Laboratory where they were sexed and placed (usually 12: 20) in 1000-cc beakers, each covered with a 20 cm section of nylon organdy anchored with the aid of paper clips to a similarly sized piece of stiff metal screen. Each of those oviposition beakers contained a small cotton plugged vial with a 5% honey-water solution for adult food. The females cemented their eggs to the nylon organdy. Organdy screens with eggs were removed and replaced daily with clean ones. Egg hatch was closely monitored in separate glass beakers covered with nylon organdy.

With the above technique it was possible to record female fecundity, percent of egg hatch, and longevity of those adults from larvae that completed their

development on various test plants.

Neonate larvae (< 8 hr. old) were transferred with a small brush to 500-cc cardboard containers (2 larvae/container) with organdy cloth lids. Fresh bouquets of leaves from the test plants were offered to the larvae in the containers. These bouquets were freshly excised foliage of potted plants that were grown outdoors at the Rome facility or of plants growing wild near the laboratory. Bouquets, held in water-filled vials plugged with cotton, were changed three or four times a week at which time larval mortality was also recorded. Dead larvae were preserved in alcohol to determine, at a later date, their instar at time of death. After 10-12 days of larval feeding 2-cm of friable soil was added to the bottom of the containers so mature larvae could pupate. Within 1 or 2 days post-emergence, adults were transferred to oviposition beakers, as described above.

It was not possible to conduct one large experiment with all of the test plants because we never had, at one time, a large number of neonate larvae and sufficient plant material from all the test plants to make large numbers of bouquets. Thus, a sequence of 6 tests were conducted. The number of containers per test plant (replicates) varied (5 to 25) in these tests.

Larval Development: Two additional studies were conducted to generate pre-liminary information on the rate of development of larvae under laboratory conditions. Some information on the number of larval instars was also gleaned from these studies. The first study was set-up on June 1, using eggs from 4 feral females captured on May 26 at Ponte Milvio. The study was started with 130 neonate larvae (2/container). Rearing containers were transparent plastic cylinders (Dia. 5 cm; length 11 cm) for 15 days, then they were substituted with 500-cc cardboard containers holding 2-cm of soil. Foliage from C. arvensis growing wild near the laboratory was offered to larvae as fresh bouquets (changed 3 or 4 times/wk). Every Monday, Wednesday, and Friday, until the larvae started to pupate, 10 containers were randomly selected and one larva per container was removed and preserved in alcohol. Laboratory air temperatures were 22-27°C during this study.

The second study was set-up on June 21 and 22, using 2 feral females captured on June 15 at Ponte Milvio to supply eggs. One-hundred neonate larvae (2/500-cc cardboard container) were used to start this study. Four containers were

randomly selected every 2-days and the 8 larvae were removed and preserved in alcohol. Laboratory air temperatures were 25-32°C during this study. Other details were similar to those previously described.

### Results -

Host Specificity Tests: Table 1 summarizes much of the results from the 6 tests. In this laboratory study larvae of  $\underline{T}$ .  $\underline{luctuosa}$  fed and developed on the 4  $\underline{C}$ . arvensis biotypes and on 9 species of  $\underline{Calystegia}$ . The percentage of larvae completing their development was  $\angle$  34% on  $\underline{C}$ .  $\underline{stebbinsii}$ ,  $\underline{C}$ .  $\underline{fulcrata}$ , and  $\underline{C}$ .  $\underline{polymorpha}$ , but percentages were considerably higher (56.25% to 90%) on the other 6 species. Three larvae completed their development and pupated (one on Baker; two on Garnet) on the sweet potato varieties; most (79.6%) of the larvae died before reaching their second instar.

We obtained some indication of the number of days spent as larvae on each of the test plants, even though data was not collected on a daily basis. The reader can refer to Table I for the data on larval development, adult emergence and longevity, and egg viability. It is worth mentioning that very few adults have emerged from larvae that completed their development. At the time of this writing (late-December) we are still holding viable pupae in the laboratory to see if adult emergence will occur in spring or summer of 1983.

Because of the high percentage of "pupal diapause", we assembled Table 2 which shows more clearly the ratio of non-diapausing to diapausing forms from each of the tests conducted in 1982. Information concerning the number of progenitor females used and their collection dates, and parasitoid emergence, is also given in Table 2. Identification of the tachinid parasitoids has not been obtained but we surmise that host larvae either ingested tachinid eggs or came in contact with planidia. Eggs or planidia were deposited by tachinids on the outdoor potted plants which served as a source of plant material for most of the laboratory tests.

<u>Larval Development:</u> Figure 1 shows the mean head capsule widths of larvae reared under the two temperature conditions. Plotting these widths against time shows that larval development was faster when temperatures were  $25-32^{\circ}$ C. When laboratory air temperatures were  $25-32^{\circ}$ C some larvae reached the 5th instar stage after 11 days of feeding. When temperatures were  $22-27^{\circ}$ C the first 5th instars were seen after 14 days of larval development.

The frequency distribution of head widths was multimodal (Figure 2). We tentatively conclude from this small data base that Tyta luctuosa has 5 larval instars. It will be necessary to sacrifice larvae on a daily basis (not every 2-3 days as we did) to generate a meaningful frequency distribution of head capsule widths.

# Aceria sp., Methods -

Host Specificity Tests: By feeding on the upper surface of <u>C</u>. arvensis leaves the mites cause the leaf edges to curl inwards and enclose them within the distorted leaves. Using information provided by Dr. Rosenthal we were able to locate an infestation of this mite. They were found in galled leaves of <u>C</u>. arvensis growing in a shady eucalyptus grove about 10 km north of Rome. First collected on July 14 at this site, we subsequently returned at least once in each of the remaining months in 1982 to collect mite-infested leaves. Galled leaves were collected from this site the same day that host specificity tests were set up at the laboratory. Galled leaves were transported to the laboratory in an ice-chest. Back at the laboratory a few galled leaves were

pried open with forceps under a dissecting microscope to check for the presence of mites.

Stems bearing 5 to 10 undisturbed galled leaves were attached with short strands of thin flexible wire to rapidly growing test plants in pots (sown in pots in March and April). These infested galls were attached to plant species in the spectrum listed above for the <u>T. luctuosa</u> study, minus the Stockton and Yreka <u>C. arvensis</u> biotypes, <u>I. batatas</u> "Baker" variety, and <u>Calystegia</u> atriplicifolia. The aim was to determine the ability of this eriophyid to gall leaves and develop on these closely related plants.

Four tests were conducted outdoors on a covered porch along the north side of the Laboratory. Test plants were in pots of various sizes (10-22 cm diameters) but all appeared to be healthy and growing vigorously at the onset of each test. A distance of 30-40 cm separated pots. Tests were initiated in July and August when daytime temperatures and relative humidities under the

porch were 10-31°C and 30-90% RH, respectively.

Tests were routinely run for 3 weeks during which time weekly observations were taken on the condition of each plant. After 3 weeks some plant material on each plant was removed and examined under a microscope to detect for the presence of living mites (also spermatophores and eggs but we were unable to differentiate between the two). After 3 or 4 weeks we found that two-spotted spider mites and a powdery mildew developed to such an extent on some of the test plants that the ability of the eriophyid mite to gall new leaves seemed to be impaired. Nonetheless, we continued to make observations on some of the test plants after the 3 week test period (well into December on some) and to make note of the gall formations.

Given below in the Results section are additional experimental details for

each test.

### Results -

Host Specificity Tests: Test 1 was started on July 15. The experimental design was a  $4 \times 4$  Latin square with 2 experimental units per square, these being 2 potted plants of the same species of biotype (we attached galled leaves to only one of these).

Test Plants - Test 1	No. Plants Infested	No. Infested galls
Convolvulus arvensis Rome biotype Ipomoea batatas UC779 Ipomoea batatas Centennial Calystegia macrostegia	4 4 4 4	4 <sup>1</sup> / 0 0 4 <u>1</u> /

 $<sup>\</sup>frac{1}{2}$  Galls were formed within one-week on these plants.

After 2 weeks one of the uninfested  $\underline{C}$ .  $\underline{macrostegia}$  plants was infested with mites. We suspect that the mites colonized this plant via passive air dispersal

from one of the nearby infested plants.

Test 2 was also started on July 15, but it was conducted in an area of the porch separate from Test 1. The potted plants were arranged in a randomized pattern. The test plants were  $\underline{C}$ . arvensis Rome biotype and  $\underline{C}$ . arvensis Texas biotype; 3 pots of each. Leaf-galling occurred on the 3 plants of the Rome biotype and on 2 plants of the Texas biotype.

Test 3 was started on August 3. The experimental design was a randomized complete block with 5 replicates of each of the 12 test plants.

Test Plants - Test 2	No. Plants Infested	No. Infested Galls
Convolvulus arvensis Rome biotype Ipomoea batatas Painter Ipomoea batatas Garnet Ipomoea batatas Jewel Ipomoea batatas Jersey Calystegia purpurata Calystegia fulcrata Calystegia occidentalis Calystegia subacaulis Calystegia macrostegia Calystegia collina Calystegia longipes	5 5 5 5 5 5 5 5 5 5 5 5 5 5	5 0 0 0 0 5 1 5 2 5 2 5 0 1/3

A few living mites were found on one plant during the microscopic inspection but leaf-galls were not formed.

Test 4 was started on August 24. There were 4 test plants with 3 or 4 replicates per plant. The plants were arranged in a randomized pattern.

Test Plants - Test 4	No. Plants Infested	No. Infested Galls
Calystegia stebbinsii Calystegia malacophylla Calystegia polymorpha	4 4 3 3	4 4 0 1

# Concluding Remarks -

In the mite tests we did not attempt to rate the level of leaf-galling per plant, believing that this would be inappropriate because plants of different ages were used (they all supported large amounts of foliage, however). We tried to run a late-summer test with uniformally aged and sized plants ( $\leq$  1 month old) but the plants were never vigorous enough to run this test.

What is apparent, however, from our work is that the Italian mite can gall the leaves of at least 8 species of potted North American <u>Calystegia</u>; leaf distortion was severe on 5 of these 8 species in the outdoor tests. To this we can add that a fungal pathogen (perhaps <u>Erysiphe convolvuli DC ex. St. Amans but identity not confirmed) readily attacked potted <u>Calystegia spp. (subacaulis, purpurata, fulcrata, and macrostegia)</u> and potted <u>C. arvensis.</u> Further, the mites continued to form galls on those potted plants that did not die by latesummer and fall. Indeed, on December 27 mites could still be found inside bud</u>

leaf-galls of potted C. arvensis, Calystegia longipes, and C. stebbinsii. Because of the concern about possible injury to wild native North American plants by biological control agents one has to be concerned about the results presented herein, and hence the future potential of our Italian populations of Tyta luctuosa and Aceria sp. as biocontrol agents of C. arvensis in North America.

In 1983, studies will be conducted at the Greek Substation to determine the ability of an eriophyid mite found on C. arvensis in Greece to form galls and develop on selected species of North American Calystegia. These tests will be conducted with potted plants under the close supervision of Dr. Sobhian.

The identity of  $\underline{T}$ .  $\underline{luctuosa}$  was confirmed by Dr. R.W. Poole, USDA-ARS, IIBIII, Beltsville, Maryland. The identity of the mite ( $\underline{Aceria}$  sp.) is a tentative one, and was provided by Professor G. Nuzzaci, Istituto di Entomologia

Agraria dell'Universita' di Bari, Italy.

HOC+ 01										
Tested	No.of Larvae	No.of larvae Completing	Days as <sub>1</sub> / Larvae 1/	Days as Pupae 1/	Emerg	nce	Adult Longevit in Days	gevity $\frac{1}{2}$	No.9 laying	% viable
	lested	<pre>% Pupating(%)</pre>			No.¥	No.ô	<b>O</b> +	\$0	viable eggs	eggs
Convolvulus arvensis Rome, wild plants	30	20(66.6)	14-17	13-15	5	7	7-22	7-15	2	100.0
Convolvulus arvensis Rome, potted plants	===	101 (90.9)	11-18	12-39	12	11	4-26	6-20	<sup>6</sup> / <sub>2</sub> / <sub>6</sub>	85.9
Convolvulus arvensis Texas	16	16(100.0)		20-42	m ·	4		9-22	2	86.0
Convolvulus arvensis Yreka	91	14(87.5)	12-15	9-15 13-39	4 %	- ~	4-11 10-11	13 8-18	m ~	9.19
Ipomoea batatas Painter	46	. 0	•		1 1	. 1	-	2	<b>.</b> 1	
batatas	01	,0			1	t			1	
batatas	72	1(1.4)	31		0	0			•	
Ipomoea batatas Centennial	20	00		•	\$ p	1 1			1	
	10	0			1				1 1	-
	46	2(4.4)	26-29		0	0			1	84
Calystegia macrostegia	25	18 (72.0)	12-15	12-34	က	2	7-12	9-16	က	86.0
	00	0.06)6	15-24		0	0			-2/	•
Calvetegia fulcrata	30	7(23.3)	21-27	18-27	4 0	<u> </u>	6-22	ر د ر	3 <u>7</u>	100.0
	16	13(81.3)	14-17	11-34	n C	2 4		11-0	ו מ	9.23
polymorpha	12	1(8.3)	27		0	0				
[-	12	4(33.3)	21-23		0	0			1	
Calystegia longipes	10	11(68.8)	14-16		0	0			ı	
	12	0			1 1	1 1			1	
	16	9(56.3)	21-25	13-18	. 4	က	4-24	9-1	14/	100.0
		•								

Because observations were taken 3 or 4 times per week the data in these columns are given as the approximate lower and upper limits.

Three females were not mated.
One female was not mated.
Two females were not mated. 21814

Table 2. Data on Adult Emergence, Parasitoid Emergence, and the Number of Diapausing Pupae in Tyta <u>luctuosa</u>. Rome Laboratory Studies, 1982.

Test No. and Host Plant	Source of Eggs	No.Larvae Completing Development on Each Host & Pupating	No.Adult Em	erging o <sup>d</sup>	Tachinid Parasitoid Emergence	No. of Diapausing Pupae
Test 1	2 Feral 9					
Convolvulus arvensis,,,	5/26	6	2	3	1	-
Rome biotype, wild / Calystegia macrostegia Calystegia purpurata		4 9	:	2 -	-	2 9
Test 2	4 Feral 9 collected 5/26	ş.				
Convolvulus arvensis 1/Rome biotype, wild	0,20	38	2	5	2	29
Test 3	2 Feral 9 collected 6/15	·:				
Convolvulus arvensis Rome biotype	0, 10	15	3	4	<b>-</b>	8
Convolvulus arvensis		14	4	1	-	9
Stockton biotype Convolvulus arvensis		16	3	4	-	9
Texas biotype Convolvulus arvensis		14	3	6	-	5
Yreka biotype Calystegia macrostegia Calystegia fulcrata		14	3 3	5	-	, 6 -
Calystegia occidentalis Calystegia subacaulis		13 11	1 2	5 7	-	7 2
Test 4	2 Feral <sup>9</sup> collected 6/15					
Convolvulus arvensis Rome biotype, wild 1/	3713	30	5	1	•	24
Test 5	1 \$\text{Laboratory}\ \text{reared(Test 1)}\ \text{3 Feral \$\text{\$\exitt{\$\exitt{\$\text{\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\$\text{\$\$\exitt{\$\text{\$\text{\$\text{\$\text{\$\text{\$\text{\$\$\}\$}}}\$}}}}}}}}}}}}}}}}}}}}}}}}}}}}}					
Convolvulus arvensis	collected 7/7	44		1	1	42
Rome biotype, Calystegia subacaulis		45	1	3	· -	41

le 2. Continued

Convolvulus arvensis	2 4 Laboratory reared (Test 4)	13	2	1	-	10
calystegia malacopylla ystegia longipes ystegia collina Calystegia stebbinsii		3 9 11 0 4	1 4	3 -	-	2 2 11 - 4
rest 7	1 P Laboratory reared 8/4(Test 3)	11/	6	2	-	3
Calystegia atriplicifol		0	-	•	-	-
Convolvulus arvensis	3 Feral 4 collected 8/17	17	1	2	-	14
Rome biotype, wild / lystegia subacaulis	:	2	3	5	2	2

This plant material was obtained from  $\underline{C}$ . arvensis growing in or near the weed garden at the Rome Laboratory.

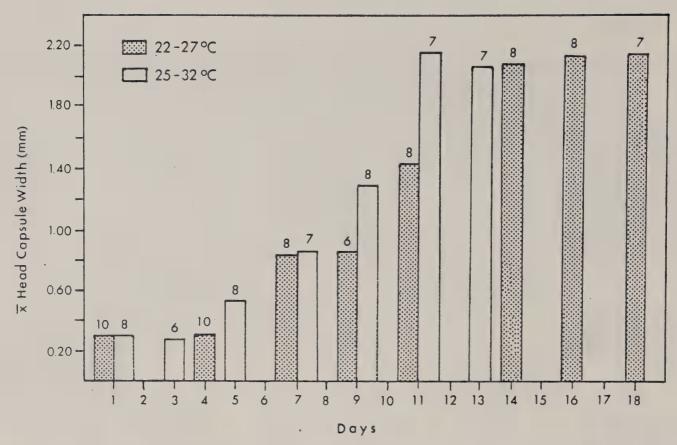


Fig. 1. Mean head capsule width measurements of <u>Tyta luctuosa</u> larvae reared under two temperature conditions and sacrificed every 2-3 days. Numbers at top of histograms denote the numbers of larvae measured.

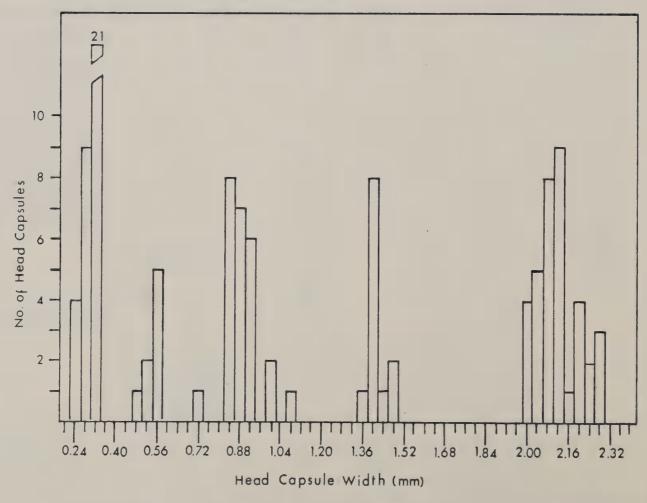


Fig. 2. Frequency histogram of head capsule widths for 116 larvae of Tyta luctuosa.

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Rumex crispus / 1.

S.L. Clement, Cristofaro, Hostettler, Mimmocchi

In March Dr. Clement visited the CSIRO Biological Control of Weeds Laboratory, Montpellier, France, to learn more about the involvement of this laboratory in the biological control of Rumex spp. (primarily pulcher). The two people working full-time on the Rumex project at Montpellier are Dr. John Scott (a Western Australian scientist) and his technician.

Shortly after returning to Rome, Dr. Clement sent a trip report to USDA people in Albany, Beltsville, and Stoneville. A decision was ultimately made to de-emphasize the Rumex project at the Rome Laboratory. The Rome Laboratory will continue to supply Neal Spencer at Stoneville with eggs of Pyropteron (=Bembecia) chrysidiforme (Esper) (sensu Naumann)(Lepidoptera: Sesiidae).

# Pyropteron (= Bembecia) chrysidiforme

Adult Collections and Field Observations: In late April about 25 roots were dug up from two sites (L'Aquila and Casalotti) known to harbor populations of this insect. These roots were transplanted into large clay pots and placed in screened outdoor cages to retain emerging adults. From these roots 5 sesiid adults (3 $^{\circ}$ ; 2 $^{\circ}$ ) and a few adults of a tachinid parasitoid emerged between late-May and mid-June. Unfortunately, all the eggs obtained from these females were infertile. Moreover, 3 feral females, captured while flying around  $^{\circ}$ Crispus in the lab garden, laid only infertile eggs after being placed in holding cages.

In mid-March, 88 non-infested (> l yr old) R. crispus plants from the Casalotti site were transplanted in the lab garden to determine if they would attract ovipositing females during the summer. The objective was to try to establish a small resident sesiid population in the garden that would yield fertile eggs in 1983 for shipment to Stoneville. The plants were attractive to adults since 5 adults were observed flying around the Rumex plants between 12 Noon and 1:30 PM on 3 days (1 on May 31, 1 on June 4, 3 on June 10). Fertile eggs were laid on some of the Rumex in the lab garden because 8 roots dissected on October 22 yielded 2 sesiid larvae. How many of the remaining 80 roots contain larvae is unknown but they will be caged next May to capture any emerging adults.

Larval Collections: Several sites were sampled with the aim of locating good natural infestations of P. chrysidiforme. Rumex crispus grown in the laboratory garden was also sampled. Excluding the Laboratory garden, the sites ranged in size from 0.25 to 3.0 hectares and were non-cultivated field supporting a variety of weeds, except the Torrimpietra (hay field) and Settecamini (fruit orchard) sites which were cultivated. The two L'Aquila sites (Abruzzo) were in the Appennine mountains (about 650 m above sea level), while the rest were in the Rome area (<100 m above sea level). Rumex crispus plants were selected at random on any given sampling date and returned to the Rome Laboratory where their roots and stems were dissected. Besides sesiid larvae, records were kept of the number of buprestid beetle larvae in roots (Capnodis tenebricosa (Oliv.); identity not confirmed) and the number of weevil adults (Lixomorphus ocularis Fabricius) in stems. Table 1 lists the locations, sample dates and sizes, and some relevant biological data on the sesiid and buprestid. Eight adult L. ocularis were found in stems (one/stem) at Casalotti: 2 dead adults

on February 25; 3 dead adults on March 22; 2 dead on April 22; and one alive on November 15. Larvae of this weevil complete their development in the roots of R. crispus during the summer, with the adults emerging in the fall.

The collective data on head capsule widths of P. chrysidiforme in Table 1 and Figure 1 suggests it completes most, if not all, of its larval development between early summer (June to early-July when eggs are laid) and late-fall (early-November for example). At Casalotti, for example, the head capsule widths on February 25, March 22, and November 15 were similar (based on 95% C.I. Figure 1). We do not know how many larval instars this insect has but the head capsule widths depicted in Figure 1 are of late instar larvae.

Seed Insects: Seeds of Rumex were collected by Neal Spencer and Niklaus Hostettler from Italy and Germany in 1981 and held in outdoor cages at the Rome Laboratory. The exact number of seeds collected is unknown but is numbered in the thousands. On May 8, 1982, 500 seeds from each collection were randomly selected and placed in gelatin capsules (mostly one/capsule). The first insects emerged on about May 26 and the last in late-June. Every two or three days during this interval newly emerged insects were aspirated from inside the cages which contained the seeds not placed in capsules; these were placed in alcohol vials. All seed insects were sent in late-November to Mr. Neal Spencer, USDA Laboratory, Stoneville, Mississippi. A total of 159 insects emerged, with 110 coming from the seeds collected near Mainz, Germany. Seeds from five Italian sites yielded few insects (23/site).

Table

location	- C	M	0 %	% of Roots infested with	with	
	Sampled	roots 1/ examined	Sesiid <u>2</u> / Larvae only	Buprestid <u>3/</u> Larvae only	Sesiid and Buprestid Larvae	x (+SD) no. sesiid larvae in roots with sesiids.
Casalotti	Feb. 25	20	35% (7)4/	10% (2)	%0	1.57+0.79(11)
Torrimpietra	Mar. 5	21		33.3%(7)	%0	
Settecamini	Mar. 5	12		8.3%(1)	%0	2.33+1.21(14)
L'Aquila #1	Mar. 19	21		9.5%(2)	14.3%(3)	$1.33 \pm 0.49 (16)$
L'Aquila #2	Mar. 19	6	33.3%(3)	(0) %0	%0	1.0 +0 (3)
Casalotti	Mar. 22	20		20% (4)	5% (1)	1.0 +0 (6)
Settecamini		8	. 0	0	12.5%(1)	
Via Palombarese		20	0	0	0	1
L'Aquila #1	Apr. 21	42	23.8%(10)	28.6%(12)	14.3%(6)	1.25+0.58(16)
Casalotti		25	20% (5)	8% (2)	0	$1.20\pm0.45(6)$
Torrimpietra		20	0	0	0	1 .
L'Aquila #1	May 18	40		2.5%(1)	~.	<i>د</i> ٠
Rome Lab Garden		6	22.2%(2)	11.1%(1)	0	
Rome (potted plants)	Nov. 5	45		2.2%(1)	0	$1.0 \pm 0$ (2)
Casalotti	Nov. 15	15		~	<i>د</i> ۰	100
Via Cassia	Nov. 15		,	1	,	16.0 (1)
Via di S. Alessandro		12	50.0%(6)		33.3%(4)	1.50+0.71(15)

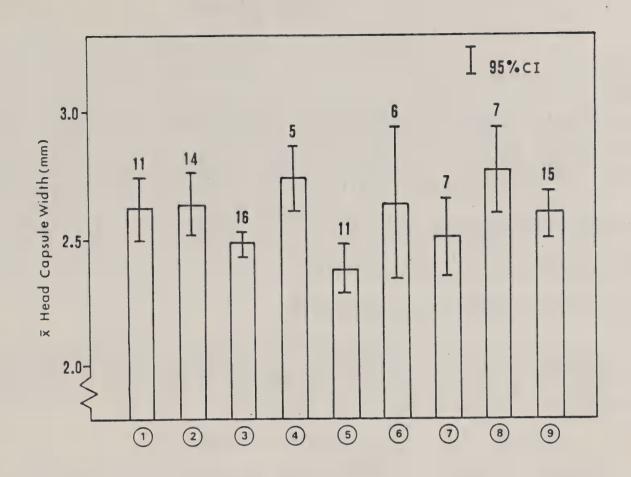
Most plants removed from field for dissection were visibly stressed.

Pyropteron (=Bembecia) chrysidiforme (Esper) (sensu Naumann). Dead larvae were (found in some instances) taken into account in compiling the statistics. 2/

Capnodis tenebricosa (Oliv.) (ID not confirmed) 3/

(n) 4/

x (+SD) no. buprestid larvae in roots with buprestids.	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
Head capsule width (x̄+SD) of sesiid larvae	2.62+0.19mm (11) 2.64+0.21mm (14) 2.49+0.09mm (16) 2.21+0.10mm (3) 2.75+0.09mm (5) 2.68 mm (1) 2.37+0.14mm (1) 2.52+0.16mm (7) 2.52+0.16mm (16) 2.66+0.14mm (16) 2.61+0.17mm (15) 2.61+0.17mm (15)



	Locality	1982 Collection Date
1	Casalotti	February 25
2	Settecamini	March 5
3	L'Aquila	March 19
4	Casalotti	March 22
5	L'Aquila	April 21
6	Casalotti	April 22
7	L'Aquila	May 19
8	Casalotti	November 15
9	Via S. Alessandro	November 22

Fig. 1. Histograms of  $\bar{x}$  head capsule widths of larval (instar(s) unknown) of Pyropteron chrysidiforme. Bars are 95% confidence intervals. Number at top of each bar is the sample size.

Galium mollugo//
SL Clement, Cristofaro

At the request of entomologists in the USDA Beneficial Insect Introduction Laboratory, Beltsville, we initiated a search for biological control agents of bedstraw, Galium mollugo L. (Rubiaceae), a persistent weed in pastures, hayfields and lawns in upstate New York and New England. However, as suggested by Dr. Suzanne Batra, Beltsville Laboratory, priority was given to locating infestations of the arthropods Schizomyia galiorum Kieffer (Diptera: Cecidomyiidae) and Eriophyes galii Nalepa (Acarina: Eriophyiidae) on G. mollugo. Our surveys were conducted within a 100 km radius of Rome. A survey of northern Italian and central European areas that are climatically similar to the New England states will have high priority in 1983.

### Survey Results and Field Observations

Twenty-one field trips were made; the first on June 1, the last on December 10. Infestations of a cecidomyiid flower bud gall-fly, a cecidomyiid stem gall-fly, a leaf-rolling eriphyid mite, leaf feeding larvae of a moth species, adults of a large chrysomelid beetle (probably a leaf feeder), and a small black hemipteran (may be a seed feeder) were located.

## Schizomyia galiorum Kieffer (Diptera: Cecidomyiidae)

Identification of this flower bud gall-fly, was confirmed by Dr. R.J. Gagne, Systematic Entomology Laboratory, USDA, IIBIII, Beltsville, Maryland. Galls of this fly were easily found in June, July, and August at all of the sites we surveyed, including those in Rome and those higher elevation sites (from 500 to 900 m above sea level) in the Appennines Mountains (Carsoli, Abruzzo).

Considerable effort was spent trying to establish a colony of this fly at the Rome Laboratory. A nucleus of > 700 galls, which were collected in June (latter half only), July and August, were used in an attempt to rear-out large numbers of adults. Little progress was made, however, because of high levels of parasitization and because our rearing technique was unsatisfactory. By collecting galls earlier in the season in 1983 we hope to obtain large numbers of unparasitized larvae; this approach should produce enough adults to proceed with preliminary host plant specificity studies.

Stem gall fly (Diptera: Cecidomyiidae)

The stem galls of a cecidomyiid fly were easily found at several sites around Rome and in the Appennines Mountains (Abruzzo) in June, July, October and November. We assume that the fly responsible for these galls on G. mollugo is Geocrypta galii, but its identity has not been confirmed by a cecidomyiid specialist. This insect was not studied in 1982 nor were attempts made to establish a laboratory colony. This insect will receive more attention in 1983.

Leaf-rolling eriophyid mite (Acarina: Eriophyidae)

A leaf-rolling eriophyid mite was first discovered in the young leaves of  $\underline{G}$ .  $\underline{mollugo}$  on July 27 at Carsoli (Abruzzo) and persisted at this site to early  $\underline{December}$ . Mites were also consistently found during the last half of summer and in the fall at a site in Villa Ada, Rome. The infestations at both sites were relatively "light"; leaf-roll galls were only found in small pockets (about  $0.5~\text{m}^2$ ), even though each site contained dense thickets of  $\underline{G}$ .  $\underline{mollugo}$  along footpaths and hedgerows. Galled plants were shaded by taller vegetation and were not visably stressed at either site.

Mites will transfer to and gall potted <u>G</u>. <u>mollugo</u> (Italian biotype) plants when they are placed among infested plants in the wild. In one instance, leaf-rolling became much more prevalent on one potted plant after we removed it from the field in mid-October and transferred it to the quarantine room (20°C; 50-80%RH) at the Rome Laboratory. Leaf galling steadily increased on this potted plant during the 3 weeks it was held in the quarantine room.

We have had some success in manually transferring mites from naturally infested plants to uninfested potted plants (Italian <u>G</u>. <u>mollugo</u>) and getting them to establish on these plants in the laboratory. We will continue to work on this aspect so that host specificity tests can be run, first using the U.S. <u>Galium spp</u>. sent to Rome.

Criocoris crassicornis (Hahn) (Hemiptera: Miridae)

Identification of this mirid was provided by Dr. T.J. Henry, Systematic Entomology Laboratory, USDA, IIBIII, Beltsville, Maryland. A series of adults were collected on June 28, Lake Bracciano (near Rome), as they were in the process of searching out seeds of <u>G. mollugo</u>. A literature survey in 1983 may provide information on the ecology, feeding habits, and oviposition behavior of this insect.

Catarhoe rubidata (Denis & Schiffermüller) (Lepidoptera: Geometridae)

Identification of this geometrid moth was provided by Dr. D.C. Ferguson, Systematic Entomology Laboratory, USDA, IIBIII, Beltsville, Maryland, who supplied the following information: "Occurs throughout most of Europe. Known to feed on Galium, but there may be other hosts. Would need to be checked out thoroughly in European literature. No best reference that I know of, but host records have undoubtedly been published somehwere, In earlier literature known variously as Cidaria rubidata, or Euphyia rubidata Schiff.".

We first found leaf feeding larvae of this insect on September 15 when they turned up on potted  $\underline{G}$ . <u>mollugo</u> in a greenhouse at the Rome Laboratory; these plants came from a site (gully along Via Vallerano) near the Laboratory. A survey of the European literature will be conducted in 1983 so a judgement can be made about the worth of this insect as a biological control agent.

## <u>Timarcha</u> sp. (Coleoptera: Chrysomelidae)

Adults of a rather large (about 15 mm in length) black chrysomelid beetle have been consistently collected in the Rome area on <u>G. mollugo</u> between November and late-December. This beetle is a species of <u>Timarcha</u>, an archaic genus comprised of apterous species, many of which feed basically on herbaceous Rubiaceae including <u>Galium spp.</u> Copulation was observed in the wild and under laboratory conditions in December. After completing a literature survey and securing the advice of chrysomelid beetle specialists we will be in a better position to make a preliminary assessment of the worth of this insect as a biological control agent.

#### Conclusion

It should be clear that Rome Laboratory personnel have a sufficient number of potential biological control agents to scrutinize in 1983. Emphasis will be given to: 1) Colonizing as many potential agents as possible in the laboratory, placing emphasis on the aforementioned arthropods, 2) Conducting preliminary host plant specificity and biological studies, 3) Using literature and taxonomic specialists to compile as much information as possible about the bionomics of the above arthropods, and 4) Cooperating with Beltsville entomologists. Surveys of more central European areas are also planned.

Abutilon theophrasti //
Cristofaro, Clement (Rome)

At the request of the USDA Stoneville Laboratory a general survey was made around Rome to locate infestations of velvet leaf, <u>Abutilon theophrasti</u> Medicus (Malvaceae). There is an interest in determining the potential for biological

control of this serious weed of field crops.

With the help of Italian weed scientists, small but spreading infestations were located in a field corn production area (Maccarese) near Rome. Plants at five Maccarese sites (within a 60 km² area) were examined closely for insect damage on eight days between June and August. Although no detectable insect damage was noted at four sites, the seed capsules of the 15-20 plants at one site yielded large numbers (ca. 250 larvae) of a lepidopterous predispersal seed predator throughout the three month collection period. Several larvae were reared out in the Rome Laboratory. A series of the moths were sent to the Insect Identification and Beneficial Insect Introduction Institute (IIBIII), Beltsville, MD., for possible identification. Dr. R.W. Hodges, Systematic Entomology Laboratory, USDA, IIBIII, Beltsville, Maryland, identified this insect as a tortricid (Olethreutinae) moth. Dr. R. Sobhian took a series of the moths to Dr. Kasy, a specialist at the Natural History Museum, Vienna, Austria, who identified the species as Crocidosema plebejana Zell. (Lepidoptera: Tortricidae). Host records for this insect are plentiful: Malva, Malvastrum, Hibiscus, Abutilon, Althaea, Gossypium. This insect is no longer under consideration as a potential biocontrol agent.

Italian weed scientists are concerned about  $\underline{A}$ . theophrasti since its importance as a crop weed in Italy is increasing. This was discovered by Dott. Massimo Cristofaro during the course of his July 13 visit with Dott. Giuseppe Zanin, Center for National Research (C.N.R.), University of Padova. During his trip to Padova, Massimo surveyed small stands of  $\underline{A}$ . theophrasti in Veneto and Lombardia, but did not find any insect damaged plants. A fungus has been observed by Dott. Zanin to attack the apical tips of  $\underline{A}$ . theophrasti. Additional

details are not known.

# R. Sobhian, Thessaloniki, Greece

Abutilon theophrasti Medicus. This plant is not common in northern Greece, at least within the surveyed area (ca. 100 km radius) around Thessaloniki. A good infestation was found near the Thessaloniki airport on October 18. When these plants were checked for phytophagous insects and diseases 4 different lepidopterous larvae were found feeding on the plant. Three of these were feeding in or on the flower heads; the fourth was a leaf feeder. The leaf feeder did not accept cotton when offered bouquets of leaves in the Laboratory. Several of the larvae pupated and were left in Thessaloniki to emerge in the spring. Some larvae of each species were taken to Austria for possible identification.

Insects shipments from Rome Lab.

Host Weed(s) * insect ** pathogen	Location	No. Stage Date	Shipping Method	Receiving Location
Euphorbia esula *Chamaesphecia empiformis	St.Polten, Austria	100 roots March 23	Airfreight	Beltsville,MD
*Aphthona flava	S.Rossore,I	1980 Adults June 23	Airfreight	Regina, Canada
*Oberea erithrocephala	S.Rossore,I	367 A June 22	Airfreight	Albany, CA.
п	S.Rossore,I	692 A June 29	Airfreight	Albany, CA.
п	S.Rossore,I	524 A July 6	Airfreight	Albany,CA
п	S.Rossore,I	265 A July 14	Airfreight	Albany, CA
Euphorbia lucida Euphorbia cyparissias * Lobesia euphorbiana	Bosco Mesola,I	160 Lärvae August 27	Airfreight	Regina, Canada
Euphorbia virgata **rust	Tulcea, Romania	August <sup>2</sup> 4	APO	Frederick,MD
Cirsium arvense * Altica carduorum	Rome,I	400 A April 21	Airfreight	Beltsville,MD
* <u>Urophora</u> cardui	Wien,Austria	3000 galls April 21	Airfreight	Albany,CA
**rust	S.Rossore,I	June 29	APO	Frederick,MD
Rumex crispus * Pyropteron chrysidiforme	Rome,I	142 eggs June 2	APO	Stoneville, MS
II II	Rome,I	220 eggs June 15	Airfreight	Stoneville,MS
Centaurea repens **pathogens	Turkey	August 27	APO	Canada
п	Turkey	August 27	APO	Zurich,Switzerla-

## Publications

A. Rizza, E. Colonnelli, P. Pecora. 1982. Notes on the Biology, Taxonomy,
Distribution and Host Records of <u>Ceutorhynchus</u> (<u>Neoglocianus</u>) <u>Maculaalba</u>
(Herbst) (Coleoptera, Curculionidae). Fragm.Entomol.Roma 16(2): 259-267.

Travels 1982	
Jan. 6	Dunn to Florence to consult with Prof. Arrigone for <u>Centaurea</u> identification.
Mar. 28-31	Clement to Montpellier'to confer with CSIRO people in order to coordinate the Rumex program.
Apr. 5-9	Clement to Delemont to confer with CIBC people.
Apr. 27-30	Pecora to Florence and Pisa to survey Euphorbia.
May 10-11	Pecora to Pisa to survey <u>Euphorbia</u> .
May 25-28	Pecora to Pisa to survey <u>Euphorbia</u> .
June 10-July 11	Rizza to Pisa to collect Oberea for shipment to the U.S.
June 15-19	Pecora to Pisa to take to the Rome Lab the <u>Oberea</u> collected by Rizza.
June 21-30	Campobasso to Greece to survey YST and make insect collections.
June 25-27	Clement to Pisa to take to the Rome Lab. the <u>Oberea</u> collected by Rizza.
July 1-3	Pecora to Pisa to take to the Rome Lab. the <u>Oberea</u> collected by Rizza.
July 6-7	Dunn and Clement to Salerno: field trip for $\underline{C}$ . solstitialis work.
July 12-17	Dunn to Greece to confer with Dr. Sobhian and the Agricultural Attache in Athens.
July 15-20	Pecora to Pisa, Piacenza and Bologna to collect <u>Oncochila</u> adults.
Aug.3-Sept.2	Dunn to Turkey and Greece to locate YST infestations, contact local scientists, and confer with Dr. Sobhian.
Aug. 24-26	Clement and Pecora to Bosco Mesola (Ferrara) to collect Lobesia larvae.
Sept.27-0ct.2	Pecora to France to collect Chrysomela sp.

Oct. 6-8 Rizza to Pisa to survey Euphorbia Oct. 21-Nov. 2 Rizza and Pecora to Yugoslavia, Rumania, Hungary and Austria to locate Euphorbia infestations, collect Chamaesphecia tenthrediniformis on Euphorbia and Urophora cardui galls on Cirsium arvense. Nov. 2-4 Dunn and Campobasso to Bari, Exploration for old infestations of C. solstitialis. Dec. 1-3 Pecora to Pisa to survey Euphorbia. Visitors February 24 Dr. John K. Scott, CSIRO Biological Control Unit, Montpellier France. Dr. Max Whitten, Chief, Division of Entomology, CSIRO, Canberra Australia. Dr. Ron Hennessey, International Institute of Tropical Agriculture, International Programs, Mowazi, Zaire. March 23 Dr. B.D. Perkins, Director, USDA, ARS, European Parasite Lab., Paris, France. Dr. R. Hedlund, Research Entomologist, USDA, ARS, European Parasite Lab., Paris, France. Mr. H. Hoyer, Research Entomologist, USDA, ARS, European Parasite Lab., Paris, France March 25 Mr. Nat Giacobbi, General Services Officer, American Embassy, Rome. Mr. James Rudbeck, Agricultural Counselor, American Embassy, Dott. Sandro Perini, Agricultural Specialist, American Embassy, Mr. Richard Perry, Security Specialist, American Embassy, Rome. April 2 Mr. H. Hoyer, Research Entomologist, USDA, ARS, European Parasite Lab., Paris, France. May 17 Mrs. Johanna T. Flaim, Program Services Officer, USDA, Washington, D.C. May 18 Dr. B.D. Perkins, Director, USDA, ARS, European Parasite Lab., Dr. R. Hedlund, Research Entomologist, USDA, ARS, European Parasite Lab., Paris, France. May 21 Dr. Peter Room, CSIRO, Australia. May 25 Mr. Ben Kopacz, Assistant to the Administrator for International

Activites, USDA, Washington, D.C. Mrs. Rita Kopacz, Washington, D.C.

June 18	Dr. Robert Norris, Associate Professor, Botany Dept. University of California, Davis, CA.
July 9	Dr. Don Simonet, Research Biologist, Mobay Chemical Corporation, Vero Beach, Florida.
July 12	Dr. Robert Pemberton, Research Entomologist, USDA, ARS, WR, Albany, CA.
August 5	Dr. Richard Lindquist, Dept. of Entomology, Ohio State University, Columbus, OH.
August 30	Dr. Glen Needham, Acarology Laboratory, Ohio State University, Columbus, OH.
Sept. 9	Dr. George Templeton, Dept. of Plant Pathology, University of Arkansas, Fayetteville, AK. Dr. W.B. Ennis, Agric. Res. and Education Center, University of Florida, Ft. Lauderdale, FL.
Sept. 13	Dr. Jerry Doll, Dept. of Agronomy, University of Wisconsin, Madison, WI.
Sept. 15	Dr. J.E. Henry, USDA, ARS, Rangeland Insect Laboratory, Bozeman, MT.
Sept. 28	Mr. Khoja Mahmuud Wasiq, (from Afghanistan), currently at Istituto Sperimentale per la Frutticoltura, Rome, Italy.
Oct. 20	Prof. Antonio Sparacino, Istituto di Agronomia, Universita' degli Studi, Milano, Italy. Dott. Marco Ialongo, Istituto Sperimentale Patologia Vegetale, Rome, Italy. Dott. Stefania Tedeschi, Istituto Sperimentale Patologia Vegetale, Rome, Italy. Dott. Gino Covarelli, Istituto di Agronomia, Facolta' di Agraria, Perugia, Italy. Dott. Vittorio Lo Giudice, Istituto Sperimentale per l'Agrumicoltura, Acireale, Italy. Prof. Franco Frilli, Istituto Difesa Piante, Universita' di Udine, Italy. Dott. Carlo Manaro, Universita' di Agraria, Piacenza, Italy. Dott. Dino Alberati, Istituto di Agronomia, Facolta' di Agraria, Perugia, Italy. Prof. Giorgio Domenichini, Direttore Istituto di Entomologia Agraria, Universita' Cattolica del Sacro Cuore, Piacenza, Italy. Prof. Mario Solinas, Istituto di Entomologia Agraria, Universita'di Bari, Italy. Dott. Vincenzo Girolami, Istituto di Entomologia Agraria dell'Univerista', Padova, Italy.

Dott. Pietro Catizone, Istituto di Agronomia, Universita' di Bologna, Italy.
Dott. Giuseppe Zanin, Centro per lo Studio dei Diserbanti del CNR, c/o Istituto di Agronomia, Padova, Italy.
Prof. Antonio Cantela, Centro per lo Studio dei Diserbanti del CNR, c/o Istituto di Agronomia, Padova, Italy.
Dott. Carmine Angelaccio, Istituto Difesa delle Piante, Facolta' di Agraria, Viterbo, Italy.
Prof. Ferdinando Bin, Istituto di Entomologia Agraria, Univ. di Perugia, Italy.
Prof. Gennaro Viggiani, Istituto di Entomologia Agraria, Universita' di Napoli, Portici, Italy.

- Oct. 26

  Dr. Orville Bentley, Asst. Secretary for Science and Education, USDA, Washington, D.C.

  Mr. James Rudbeck, Agricultural Counselor, American Embassy, Rome, Italy.

  Mr. Ben Kopacz, Assistant to the Administrator for International Activities, USDA, ARS, Washington, D.C.
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